RESEARCH FINDINGS OF FEW MEDICINAL PLANTS HIGHLIGHTING THE SCOPE FOR FURTHER STUDY

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Abstract. Several plants are there which are absolutely unknown to the world but have enormous medical values in curing certain diseases. This paper aims to identify the medicinal values and importance of some of those plants which can cure some diseases which are chronic as well as can be difficult to be cured in certain cases. Four such plants, viz., Alangium salvifolium, Argyriea nervosa, Hygrophila auriculata and Oroxylum indicum are chosen to detect their pharmacological values against certain diseases alongside their ayurvedic, ethnobotanical and traditional values. Their pharmacognostical characteristics are also discussed and the phytochemicals which are so far investigated by certain studies are also been discussed. Molecular studies such as docking, LC-MS, GC-MS detections of biochemical compounds are also discussed in this paper. It is seen that every plants have certain ayurvedic uses as each parts of these plants can be applied tropically or may be used as decoctions or as pastes or can be orally consumed to cure diseases. Pharmacognostical studies reveal the microscopic, macroscopic as well as the other physical factors of different plant parts. Pharmacologically these plants in certain doses are capable of curing arthritis, inflammations, sometimes can be used as anti-cancer and anti-ulcerative agents and in many other diseases including diabetes. LC-MS, GC-MS studies and other studies revealed the presence of certain phytochemicals and biochemical compounds such as flavonoids, fatty acids, esters of fatty acids, carbohydrates, amino acids and several other compounds. This paper also aims to find out the future scopes of research on these plants where scientists can work towards fulfilling it for betterment for mankind.

Keywords: arthritis, ayurveda, ethnobotany, pharmacology, pharmacognosy, phytochemicals

Introduction

Plants and their disease curing abilities go hand in hand may be since the concept of Ayurveda has come into existence during the Indus Valley Civilization back in 6,000 B.C. Even though today when the world is absolutely cruising towards advancement in every possible ways with technologies being at the helm of all aspects, a large part of the population relies on the ancient way of treating diseases in which the plants are hugely involved. There are about thousands of plants around the world which somehow or the other are medically important and are used in pharmacological industries for the production of tablets, creams and ointments to be consumed or applied tropically in a large variety of diseases. Every single part of these plants are important and are sometimes used making a paste of leaves, the powdered root or seeds are used, dry or along with water, the decoctions and the infusions are used to treat a large variety of infections and diseases from arthritis, pain to diarrhea and throat infections (Uphof, 1968). These plants have high ethnobotanical values as well as they are often used in villages to treat pregnancy complications as well as in snake bites (Rajamanickam et al., 2009). Traditional values of these plants are also too worthy to be looked down upon as making of baskets, toothbrushes (Tanwer and Vijayvergia, 2014), temporary houses and other household purposes are solved by using different parts of these plants around the

globe. These plants can be used to extract several phytochemicals and biomolecules from the oil obtained by several solvents which are often used in various molecular studies as well as in production of several cosmetics. Thus overall, they have huge market values which on the other hand boosts the economy as well. Even when the formulated encapsulated medicines are worth high in prices, these raw parts used in local remedies often cuts down the expenditure of a patient which is highly cost effective and is devoid of fatal side effects. With the current molecular studies in progress with respect to these plants, the scientists have a huge scope of detecting various other fields in which these plants can be used, be it in pharmacology or in other aspects which will ultimately help the mankind.

In this paper, four such medically important plants were selected in Alangium salvifolium, Argyriea nervosa, Hygrophila auriculata and Oroxylum indicum. The taxonomical positions, the ayurvedic aspects, the ethnobotanical and traditional uses, the pharmacognostical values, the phytochemicals investigated, the pharmacological importance, and the molecular studies so far that has been done on these plants are discussed thoroughly.

Taxonomical classification

Taxonomical hierarchy of the selected plants for study is shown in Table 1.

No.	Division	Class	Order	Family	Genus	Species	Common
							names
1	N/A	Dicotyledons	Cornales	Alangiaceae	Alangium	salvifolium	Angol, Dhera
2	Tracheophyta	Magnoliopsida	Solanes	Convolvulaceae	Argyreia	nervosa	Elephant Creeper Samudrasos
3	Tracheophyta	Magnoliopsida	Lamiales	Acanthaceae	Hygrophila	Auriculata	<i>Vayalchulli</i> Neermulli Kamtakalya
4	Magnoliophyta	Magnoliopsida	Lamiales	Bignoniaceae	Oroxylum	indicum	Sonapatha

Table 1. Taxonomical hierarchy of selected plants.

Source: Harminder and Chaudhary (2011)

Alangium salvifolium

Alangium salvifolium is a flowering plant of Cornaceae family, commonly known as Ankolam in Malayalam, Ankola in Kannada, Akola or Ankol in Hindi and Alanji in Tamil. In India, It is mostly found in dry regions in plains and low hills and also found on roadsides as small, bushy tree offering a dense canopy with a short trunk (Tanwer and Vijayvergia, 2014; Krishen, 2013; Neginhal, 2011). In india, it is found in Andhra Pradesh, Bihar, Chhattisgarh, Goa, Gujerat, Karnataka, Kerala, Madhya Pradesh, Mahar Ashtra, Odisha, Rajasthan, Tamil Nadu, Tripura, Uttar Pradesh, Uttarakhand and West Bengal (Ganeshaiah et al., 2012). The plant is harvested from the wild, especially in India, for its use in traditional medicine, succeeds in any moderately fertile, well-drained soil and requires a position in full sun (Huxley, 1992).



Figure 1. Morphology of A. salvifolium whole (A) tree, (B) leaves, (C) flower, (D) fruits and (E) seeds.

Ethnobotany

In Ayurveda, the roots and the fruits are used for treatment of rheumatism, burning sensation and haemorrhages. The root barks of *A. salvifolium* are used externally as an antidote against snake/scorpion, rabbit, rat, dog bites. In Philippines, the roots and the fruits are used for treatment of rheumatism and hemorrhoid externally (Rajamanickam et al., 2009) In Africa, a decoction of whole plant along with fruit of coconut is used to treat boils. Leaves are used to cure asthma in China (Jane et al., 2010).

In India, *A. salvifolium* has been used as laxative, antiepileptic, astringent, antiulcer, anthelmintic, emetic, anti protozoal and hypoglycemic agent. It has been reported that it is used to cure skin diseases, leprosy, scabies and as contraceptives for pigs and cattle rearing by the tribes in Kerala. The root-bark is also used to expel parasitic worms (Platyhelminthes) and other internal parasites from the body. It is used as an emetic and purgative as well (Ganeshaiah et al., 2012). The root-bark is also used in traditional medicine skin problems (Tanwer and Vijayvergia, 2014; Krishen, 2013; Neginhal, 2011).

Phytochemistry and pharmacology

The detailed pharmacognostic studies are done by Shravya et al. (2017), where the stomatal index, stomatal frequency of leaves, the vein islets and termination shows moderate to average number. The organoleptic evaluation reveals that the extracts from different plant parts are odourless and tasteless showing different colours under fluorescent light (Shravya et al., 2017). The aqueous and ethanolic extract of leaves revealed the presence of alkaloids, tannins, titerpene and steroids, whereas roots revealed the presence of phenolic compounds and flavonoids. Methanolic extracts from stems and leaves contained flavenoids, terpenoids, alkaloids and steroids. Flower juice in ethanolic extract produces phenolic glycosides, salviifosides, salicin, kaempferol, kaempferol-3-O-b-D-glucoropyronidase and Methy,1-1H-pyrimidine-2,4-dione and 3-

O-b-D-glucopyranonyl- (24β) -ethylcolesta-5,22,25-triene. Root bark contains flavonoids in extracts of benzyl and ethyl acetate.

The extracts of Alangium salvifolium showed potent antiarthritic activity by Fruends adjuvant arthritis model and the potency of the activity followed the order standard > chloroform > ethyl acetate > aqueous > petroleum ether > methanol. Steroids are reported to possess antiinflammatory property (Venkateshwarlu et al., 2011); since these phytoconstituents are found in our extracts may have contributed for exhibited anti-arthritic activity by inhibiting the inflammation due to the Fruends adjuant. Stem bark of Alangium salvifolium showed significant antimicrobial activity against all the selected strains of bacteria, fungi and yeast (Tanwer and Vijayvergia, 2014). The methanol extracts of Alangium salvifolium flowers showed a wide spectrum of antibacterial activity against both Gram-Positive and Gram-Negative bacteria (Mosaddik et al., 2000). Leaf extracts were screened against pathogenic strains of Escherichia coli, Proteus vulgaris, Bacillus subtilis, Enterobacter faecalis, Serratia marcescens and Klebsiella pneumoniae (E Natarajan et.al, 2012); against selected fungi Aspergillus niger, A. fumigatus, A. flavus, Fusarium oxysporum, Penicillum sps and Rizopus sps (Tanwer and Vijayvergia, 2014). The antioxidant activity of callus and other plant parts of Alangium salvifolium was measured using 2, 2'-diphenyl1picryhydrazyl (DPPH) free radical and superoxide radical scavenging assays. The results showed that all the parts exhibited antioxidative activity. And highest activities were recorded in 8 week old callus (Tanwer and Vijayvergia, 2014). In Nitric oxide method, alcohol extract of roots presented more antioxidant activity than aqueous extract (Jain et al., 2010). In acetic acid-induced writhing in mice, ethanolic and water extract showed significant analgesic activity, whereas the chloroform extract was found to be ineffective. Ethanolic extract of Alangium salvifolium was found to inhibit the paw edema as time and dose dependent manner when compared to standard diclofenec sodium (Tanwer and Vijayvergia, 2014). The methanol extract of plant roots has been studied for analgesic and anti-inflammatory activities in animal models. The activity was dose dependent, writhe movements were reduced in crude ethanolic extract which was comparable to diclofenec sodium (Tanwer and Vijayvergia, 2014). In excision wound model, animals treated with ethanol extract showed significant decrease in epithelization period. Significant increase was also observed in improved collagen maturation by increased cross-linking while an increase in dry granuloma weight indicated higher protein content (Karigar et al., 2010).

The seed extracts showed a significant increase in glucose tolerance. These results indicated that extracts may have the capacity to block glucose absorption. Daily administration of petroleum ether, ethyl acetate, chloroform, methanol or aqueous extracts of *A. salvifolium* for eight days starting from the first day of pregnancy showed significant abortifacient activity in comparison to vehicle treated group. It indicated that the herbal drugs may have anti-progesterone effects. Mifepristone, a competitive inhibitor that acted both at progesterone and glucocorticoid receptors, and a weak partial agonist with predominantly antagonistic activity to progesterone (Murugan et al., 2000). LC-MS studies of aqueous extracts and the organic extracts of *Alangium salvifolium* were performed where LC/MS grade isopropanol, methanol and acetonitrile. The separation chemistries for LC/MS/MS were performed using Poroshell HILIC Plus and three orthogonal Reverse Phase Coloumns. GC studies revealed flavonoids, fatty acids, sugars, terpenes and so forth. LC/MS analysis resulted in identification of 449 and 62 compounds respectively (Siddaiah et al., 2021).

Molecular aspects

A Study was conducted to evaluate Alangium salvifolium derived analgesic compounds for therapeutic drug discovery by computational approach. Literature based studies were used to explore the compounds, ligands were prepared and in silico docking analysis was performed by GOLD 4.2 (Dash et al, 2015). Upon docking analysis, salviifosides A of Alangium salvifolium was found to have interaction on COX-2 protein by obtaining highest fitness score and suggests that it could be a potent anti-inflammatory compound and it may be worth for further clinical trials. Another research was done to retrieve and draw the active phytocompounds structure and assessing its simulation anti-oxidant enzyme activities by using *chem.-sketch* software. The 3-D structures of the Phytocompounds were visualized based upon the UV, NMR spectral data along with their energy simulation studies. The antioxidant and enzyme simulation activity were evaluated in-silico using the softwares ACD labs, PyRx, RASMOL, PYMOL, Araguslab and Discovery 3.1 studio.

The plant phytochemicals showed significant anti-oxidant enzymes activity enhancer and ROS eliminator through binding to its metal domain receptor (Khan, 2019). It can be concluded that among these phytochemicals, were studied anti-oxidant enzymes metals binding domain to increase the ROS scavenging activity for the foremost time from mimic with molecular docking.

Argyreia nervosa

Argyreia nervosa is a perennial plant and a climbing vine which is native to subcontinent and numerous areas including Hawaii, Africa, the Indian and the Caribbean (Figure 2). Though it is invasive in nature, it is often used for its aesthetic and medicinal value. Its seeds are very useful for their powerful entheogenic value (Altwo 1999; Paulke et al., 2015). The botanical varieties Assmar, are A. n. var. nervosa and A. n. var. speciosa, which are used as Ayurvedic medicine. Argyreia is a genus of morning glory family with 90 species native to continental tropical Asia, Malaysia and Northern Australia (Acevedo-Rodríguez, 2005). "Argyro", referring to the silver colour at the underside of the leaf and species epithet is a reference to its conspicuous veins (Csurshes, 2009). The two most commonly cited names are 'elephant creeper", referring to its large leaves that resemble the ears of an elephant. It is often grown professionally under glass in loamy potting compost in full light, and watered freely from spring to autumn, with a balanced liquid fertilizer applied monthly, gradually reduced water in winter (Brickell, 2016). It is grown outdoors in moderately fertile, moist but well-drained soil in full sun.



Figure 2. Argyriea nervosa with (A) whole plant, (B) leaves, (C) flower, (D) fruits and (E) seeds.

Ethnobotany

A. *nervosa* leaves extract applied topically promotes healing of wounds more significantly as compared to oral application, in both normal rats and alloxan induced diabetic rats (Singhal et al., 2011) where healing is otherwise delayed. The roots and the seeds are used in traditional Ayurvedic herbal medicine in India (Kapoor, 2001). The plant is considered to be alterative, aphrodisiac, antiphlogistic, antiseptic, emollient and tonic. The root is often used in the form of a powder. It is used internally as an alternative in the treatment of conditions.

Externally, a paste of the roots is used as a poultice on rheumatic joints, boils, scorpion stings etc. (Vanila et al., 2008) and is rubbed over the body to reduce obesity. The leaves are antiphlogistic, emollient (Vanila et al., 2008; Kapoor, 2001).They are used as poultices on skin diseases and wounds (Uphof, 1968). The silky side of the leaf is applied to tumours, boils, sores and carbuncles. Some common myths associated to *Argyreia nervosa* are, Hindu astrology relates *Argyreia nervosa* with the planet mercury and suggests that a person being adversely affected by the negative effects of the mercury planet must tie the *Argyreia nervosa* root with green tag and cloth and tie it up on the upper hand on a Wednesday (Singhal et al., 2011) since mercury planet resembles that particular day according to hindu shastra. The whole plant can be used in treatment of small pox and other skin diseases through tropical application by forming a paste of the plant parts. Roots of the plant are useful as an Appetitiser, anaemia, aphrodisiac, anti–inflammatory, brain-tonic, cardiotonic, cerebral disorders (Nadkarnt, 1995), diabetes.

Traditionally the plant is used in the treatment of gonorrhea, strangury and chronic ulcers. A preparation "Fortege" made from this plant along with several other ingredients is used to cure sexual disorders in males. Another drug "Speman" consisting of several ingredients of plant material including this species, is reported to exhibit anabolic as well as androgen-like activity in mice (Saxena and Brahman, 1994), in stomach complaints, sores on foot, small pox, syphilis, dysentery and diarrhoea (Guha Bakshi et al., 1999; Nadkarnt, 1995). The leaves are used externally in the treatment of

ringworm, eczema, itch and other skin diseases (Pandey, 1996). It is also used as a local stimulant and rubefacient (Kirtikar, 1981). Seeds of Argyreia nervosa found to possess hypotension, spasmolytic (Agarwal and Rastogi, 1974) and anti-inflammatory activities (Gokhale et al., 2003). Roots of Argyreia nervosa proved the immune modulatory activity against the myeloid suppressive effects induced by Cyclophosphamide (Gokhale et al., 2002). The root was also used in appetizer, anemia, aphrodisiac, antiinflammatory, brain-tonic, cardio tonic, cerebral disorders (Krishnaveni and Thaakur, 2009; Prajapati, 2003). The leaves are also used internally to cure boils and swellings. In some parts of India, leaves and seeds are eaten (Galani et al., 2010; Meher and Padhan, 2011). It is also reported to be used for the fibres (PROTA Official Portal, 2016). The seeds of the plant can be consumed to produce psychedelic effects (Rahman et al., 2003). It is also possible to mix genuine seeds of Argyreia nervosa with that of Argyreia speciosa and convince the users that they lack essential effect (Kremer et al., 2012). A curious effect of seeds is an urge to stretch and extend limbs and muscles in arms and legs which is similar to that of consuming Lysergenic acid di-ethylamide (LSD). The fresh roots of Argyreia nervosa reveal the external visible characters like the shape, size, diameter and also the organoleptic properties. A pharmacognostic study reveals the morphological characters of the bark of Argyreia nervosa, and organoleptic, physico-chemical, fluorescence analysis of powder drug (Jeet et al., 2012a). It states that the drug can be characterized specifically on the basis of these characters. The crude powder drug, ethyl acetate and methanol extracts are investigated for the presence of different phytochemicals.

Phytochemistry and pharmacology

Roots of Argyreia nervosa in the aqueous extract showed presence of Alkaloids, acids Flavonoids and Tannins (Mujum Glycosides, Amino et al., 2010). Methanolic extract the of Argyreia nervosa showed of roots presence of Alkaloids, Flavonoids, Glycosides, Amino acids, Steroids and tannins. The seeds various ergoline as ergine alkaloids such (Halpern, 2004). A study contain reported stereoisomers of ergine to be found in the seeds at a concentration of 0.325% of dry weight. A more recent study reported presence of ergometrine, lysergol, lysergic acid and other alkaloids (Chao and Der Marderosian, 1973) that contribute to its pharmacological effects. Fatty oil from seed extracts consisted of Glycosides of Palmitic, Oleic, Streaic, Behenic, Linoleic and Linolenic acid. By GLC analysis, Myristoleic, myristic, Plamitic, oleic, stearic, linoleic, linolenic, non-adecanoic, eicosenoic, heneicosanoic, and behenic acids are obtained. Branched fatty acids such as 12-methylmyristic acid, 15-methylstearic acid. The ethanolic extract reported presence of Ergometrine (alkaloid), caffeic acid, ethyl caffeate. A 30.6 % Crude protein and a 10.4% of albumin, 8.8% globulin and Glutenin 10.6% are present as well (Padhi et al., 2013). ntriacontanol, β- Sitosterol, p- hydroxycinnamoyl octadecanolate, caffeic acid are obtained from fruit. From petroleum ether extract of leaf contained 1-triacetanol, epifriedelinol acetate, β- sitosterol. Flavenoids, Quercetin, Kaempferol. Hexane extract from roots are consisted of Tetradecanyl palmitate, 5,8- oxidotetracosan-10-one. Esters present are Stigma steryl p-hyroxy cinnamate and hexadecanyl.

Argyreia nervosa, as a whole plant and its solvent extracts covers a huge area in pharmacology with its aspects of medicinal value and uses. In an experiment conducted on rats, it shown a prophylactic treatment with leaf extracts suppressing the histopathological changes in joints and significantly reduced the Freund Complete

Adjuvant induced paw edema, Rheumatoid Factor, Erythrocyte sedimentation Rate (ESR), Lipid peroxidation and significantly elevated the Freund Complete Adjuvant, decreased Superoxide dismustase, Catalase and reduced glutathione levels as compared to arthritic rats (Alugonda et al., 2014). These results suggested that leaf extracts possess anti-arthritic activity at least in part by preventing oxidative stress. Another study evaluated the in vivo anti-arthritic effect of polyherbal formulation of selected plants such as *Polygonum glabrum, Canthium dicoccum, Ochna obtusata*, and *Argyreia nervosa* where the In vivo anti-arthritic activity of the ethanolic extract of different portions capsule formulation F4 investigated orally was assessed using complete Freund's adjuvant-induced arthritis (Satyanarayana, 2019). In complete Freund's adjuvant-induced arthritis models, the polyherbal extract formulations was seen to significantly reduce joint and paw swelling and markedly improved body weight, hematology profile, and parameters in complete Freund's adjuvant model.

Alcoholic extracts of roots showed anti-inflammatory activities against Granuloma formation in the Albino rats but not against formalin induced arthritis in rats (Srivastava et al., 1972). Ethyl acetate and methanol extract of the whole essential part from Argyreia nervosa was studied for anti-inflammatory activity on healthy wistar strain albino rats weighing 140-250 g with carrageenan induced paw edema (Jeet and Thakur, 2012). Both the extracts showed significant anti-inflammatory activities. The methanol and ethyl acetate extract of the whole aerial part of Argyreia nervosa showed positive antipyretic activity on healthy wistar rats weighing around 150-200 g (Jeet and Thakur, 2012) with usage of brewer's yeast. Aqueous extracts of the leaves shows antibacterial effects against S. aureas and E. coli, where alcoholic extracts showed antibacterial activities only against the S. aureus. (George and Pandalai, 1949). The seed has antibacterial activities against both gram positive as well as gram negative bacteria with an exception to S. aureus. (Mishra and Chaturvedi, 1978; Batra and Mehta, 1985). Other species on which antibacterial activities are shown includes, Bacillus subtilis, Bacillus cereus, Klebsiella pneumoniae, etc. (Modi et al., 2010; Basha and Sudarshanam, 2011; Mahule et al., 2012). Antifungal activities of seed oil is recorded positive against Aspergillus niger, Aspergillus flavus, C. capsici, Cryptococcus neoformans, Alternaria solani, Helminthosporium sp, Collectotrichum dematium, A. sydowi and Fusarium oxysporum with an exception to Penicillium sp. (Mishra and Chaturvedi, 1978). Roots also are identified to have antifungal activities. The extracts of the plant and fruits showed antiviral activities against the Vaccinia virus (Babbar et al., 1982).

Ethanolic and hydroalcoholic extracts of the roots showed significant analgesic activities with usage of methods as in writhing and tail flick methods in rats (Galani and Patel, 2009). Ethyl acetate and methanolic extracts of whole arieal part of the plant used on healthy albino mice and wistar rats using acetic acid induced writhing and tail immersion methods (Jeet et al, 2012b) shows significant analgesic activities. Ethanolic extract of the leaves while applied on diabetic animals for wound healing property by oral and tropical administrations, the tropically applied method was seen to be more effective (Singhal et al., 2011) compared to that of oral. Leaf flower and root extracts showed aphrodisiac activities and stimulates male sexual activities (Subramoniam et al., 2007). Product "Speman' consisted of several plant ingredients which exhibited anabolic and androgen like activity in mice with FSH synthesis, sperm motility promotion (Jaytilak et al., 1976). It can be used as a central nervous system (CNS) depressant by its root extracts in solvents such as chloroform, n-hexane and ethyl

acetate as they shows reduction of spontaneous motor activity and potentiated pentobarbital induced hyponosis in mice (Galani and Patel, 2009). Scavenging activities of the aqueous extract of the roots were seen in methods as DPPH assay, ABTS scavenging, Lipid peroxidation, Iron chelation, superoxide and antioxidant capacity. Glycosylation of Haemoglobin assay (Shreedhara et al., 2009) indicates significant antioxidant activity.

Molecular aspects

The seeds of Argyreia nervosa are considered as a natural substitute of Lysergenic Acid Diethylamide (LSD), in study Lysergenic acid amide (LSA) and LSD was compared to their potential pharmacological profiles based on the receptor binding. Initially, computer-aided in silico prediction models on receptor binding were employed to screen for serotonin, norepinephrine, dopamine, muscarine, and histamine receptor subtypes as potential targets for LSA. The screening was accompanied ergotalkaloids of Argyreia nervosa (Burm. f.) to verify, selected LSA screening results were confirmed by in vitro binding assays with some extensions to LSD wherein silico model, LSA exhibited the highest affinity with a pKi of about 8.0 at α 1A, and α 1B. Clear affinity with pKi more than 7 was predicted for 5-HT1A, 5-HT1B, 5-HT1D, 5-HT6, 5-HT7, and D2. From these receptors the 5-HT1D subtype exhibited the highest pKi with 7.98 in the prediction model. From the other ergotalkaloids, agroclavine and festuclavine also seemed to be highly affine to the 5-HT1D-receptor with pKi>8 (Paulke et al., 2013) It was concluded that since the psychedelic activity of LSA in a recent human study was weak and although LSA from Argyreia nervosa can be considered as natural exchange for LSD, LSA should not be regarded as LSD-like psychedelic drug.

In another study, green synthesis of silver nanoparticles (AgNPs) using *Argyreia nervosa* leaves extract as a potential reducing and capping agent was done. Surface plasmon resonance confirmed the formation of AgNPs with maximum absorbance at 485 nm. FTIR studies reveal that involvement of biological macromolecules of the extract in the synthesis and stabilization of AgNPs. HRTEM images showed that the size of the spherical AgNPs ranged between 5 and 40 nm with average particle size of about 15nm. The AgNPs extracted from the extract showed inhibition activity against carbohydrate digestive enzymes (α -amylase and α -glucosidase), with EC₅₀ of 55.5 and 51.7 µg/mL respectively, indicating the antidiabetic potential. The in vitro antioxidant activity of Ag NPs were evaluated in terms of ABTS {2-2'-azino-bis (3-ethylbenzothiazoline 6-sulphonic acid)} and DPPH (1,1- diphenyl-2-picryl hydrazyl) free radicals scavenging assays with IC₅₀ value of 44.3 and 55.9 µg/mL respectively (Saratale et al., 2017). The AgNPs displayed strong antibacterial activity against few food borne bacteria.

GC-MS study

A study was conducted where seedling derived nodal explants were inoculated on MS medium supplemented with Gibberellic acid and Kinetin. For GC-MS analysis methanolic extracts of different samples viz nature and in vitro raised plants were used. Maximum frequency of shoot induction and maximum mean shoot length were obtained on Kinetin. Whereas GA gave maximum mean shoot number. Indole-3-butyric acid showed maximum frequency of root induction and maximum mean root length. A large variety of compounds have been detected in methanolic extract of *A. nervosa* including

1,3,4,5-Tetrahydroxy-Cyclohexanecarboxyl, n-Hexadecanoic acid, Phytol etc. (Bharati and Bansal, 2015). The study was reported on *in vitro* protocol for *A. nervosa* and also confirmed the presence of many new therapeutically potent compounds in different *in vitro* and nature grown plant extracts through GC-MS analysis.

Hygrophila auriculata

Hygrophila auriculata, is herbaceous, medicinal plant (Gamble, 1921) that grows in marshy places and is native to tropical Asia and Africa (*Figure 3*). In India it is commonly known as *kokilaksha* or *gokulakanta*, in Sri Lanka as *neeramulli*. It is commonly found in moist places - on the banks of tanks, ditches, and paddy fields (Kshirsagar et al., 2010). *Hygrophila auriculata* is an erect, stout, branched or unbranched, annual herb growing 150cm or more tall (Burkill, 1985). Occasionally the basal part of the stem is creeping and rooting (Bentjee, 2002). Stem is a robust herb or undershrub, growing up to 80 cm tall with sparsely sub-hispidly hairy stems and whorled spines at the node (Bera et al., 2017). Flowering and fruiting occurs during the months of August-March. The plant is harvested from the wild for local use as a food and a medicine. It is cultivated in water beds in West Africa both as a vegetable and for the vegetable salt it can yield (Burkill, 1985), and is sold as a vegetable in the local markets of Sri Lanka (Mathiventhan and Ramiah, 2015).



Figure 3. Hygrophila auriculata with (A) whole plant, (B) leaves, (C) flower and (D) seeds.

Ethnobotany

In Ayurveda, its seeds, roots and panchang (pancha i.e five and ang i.e parts, means root, flowers, stem, fruits and leaves as ash burnt together) are used as a medication (Daniel, 2006). Its leaf is reported useful in a cough and anal fistula. Its seeds are useful in blood disorders. Intake of root decoction is useful in jaundice. Topical application of its leaf paste reported is useful in Prameha, lumbago and arthralgia. Its root and a whole part decoction are reported useful in rheumatoid arthritis (Gaur, 2007). Intake of Talmakhana ash along with cow urine or water is useful in inflammation (Tripathi, 2019). In India, it is commonly used as an edible vegetable in some states like Odisha, Chhattisgarh and West Bengal, and is used for the treatment of cancer, arthritis,

hepatotoxicity, inflammation, blood diseases, diabetes, fever, constipation, bacterial infection etc.

Root is used for the treatment of severe diseases as diuretics, dropsy, rheumatism, jaundice and also to kill abdominal insects and the roots have medicinal properties such as coolant, aphrodisiac, tonic, and anti-inflammatory and useful in hyper thirst, calculus, flatulence and associated disorders (Sheth et al., 2005; Shastri and Chaturvedi, 2004). The decoctions of the young leaves are taken orally for two consecutive weeks in empty stomach to treat anemia. Leaf is also used to treat for pain, poison, pretentiousness, abdominal disorders, constipation, and urinary disorders. Seed powder mixed with raw cow milk is taken in the morning for treating impotency. Dry seed powder mixed with milk and sugar is taken to cure spermatorrhoea (Bera et al., 2017) It is also used for removal of gall stones as aphrodisiac and as Tonic for anti-diarrheal and conception promoter. Efforts have been made by researchers to verify the efficacy of the plant by scientific biological screening. The pharmacognostic studies include the detailed

Phytochemistry and pharmacology

A lot of phytochemicals has been extracted from the plant from its different parts. The plant contains saponins, alkaloids, steroids, tannins, flavonoids and triterpenoids as the main components (Hussain et al., 2010). Flavenoids as apingenin-7-O-glucuronide along with traces of 7-O-glucosidase (Balraj and Nagarajan, 1982) are extracted from flowers. Two alkaloids as asteracanthine and asteracanthicine (Parashar and Singh, 1965) are extracted from seeds. Lupeol is found in stems, leaves and roots and a hydrocarbon hetricontane is found in leaves and stem (Govindachari and Nagarajan, 1957). Botulin is isolated from aerial parts (Misra et al., 2001). Luteolin and luteolin-7-O-rutinoside are found in leaves (Henry et al., 1989) and stigmasterol found in roots (Quasim and Dutta, 1967). Aliphatic esters as 25-oxo-hentriacontyl acetate and methyl 8-n-hexyltetra cosanoate are found from methanolic extract of aerial parts (Misra et al., 2001). Minerals as Fe, Co, and Cu are found and seed protein contains essential amino acids (Choudhary and Bandyopdhyay, 1980) that are similar to that of groundnut proteins.

Extracts and bioactive compounds from the plant have been found to possess antimicrobial, , hepatoprotective,, antitumour, antidiabetic, antioxidant, diuretic, antinociceptive, anti-inflammatory, antipyretic, aphrodisiac activities (Sethiya et al., 2018). The antinflammatory and antipyretic activity of the petroleum ether, chloroform, alcoholic and aqueous extracts of the leaves studied based on their effects on carrageenan-induced paw oedema and Brewer's yeast-induced pyrexia in rats respectively showed chloroform and alcoholic exhibited significant anti-inflammatory and antipyretic activities in a dose-dependent manner while the petroleum ether and aqueous extracts did not have any significant anti-inflammatory and antipyretic activities (Hussain et al., 2009). Methanolic extract of the seeds exhibited potent hepatoprotective activity against paracetamol- and thioacetamide-induced liver damage in rats (Singh and Handa, 1995). Aqueous extract of the exhibits potent hepatoprotective activity against carbon tetrachloride-induced liver damage in rats (Shanmugasundaram and Venkataraman, 2006). The anti-tumor activity of seeds against experimental hepatocarcinogenesis in rats are observed which showed that the seeds significantly affected the activities of the antioxidant enzymes, glutathione peroxidase and catalase, in a dose-dependent manner (Ahmed et al., 2001). Petroleum ether extract of the root exhibited anti-tumour activity in mice with Ehrlich ascites carcinoma and

sarcoma-180 (Mazumdar et al., 1997). The hydroalcohlic extract of the whole plant showed antitumor activity against anthracene (DMBA)-induced mammary tumors in female rats comparable with a standard drug (Paulke and Sunita, 2008).

Aqueous extracts of the whole plant significantly lowered the fasting blood glucose level and markedly improved the glucose tolerance of rats at a therapeutic dose equivalent to 5 g/kg of the starting material (Fernando and Wickramasinghe, 1991). An ethanolic extract of the aerial parts of *Hygrophila auriculata*, at a dose of 100 and 250 mg/kg body weight for 3 weeks, induced a significant reduction in blood glucose, thiobarbituric acid reactive substances (TBARS) and hydroperoxide in liver and kidney in streptozotocin-induced diabetic rats. Ethanolic extract of the aerial parts of Hygrophila auriculata possessed significant antidiabetic activity along with a potent antioxidant activity in models of diabetes (Vijayakumar et al., 2006). The plant parts used traditionally to treat inflammatory diseases for an inhibitory effect on lipid peroxidation and reported that the plant inhibited lipid peroxidation with an IC_{50} Value of 20 µg/ml (Sunil and Müller, 1999). The ethanolic extract of the leaves, stem, fruits and root of Hygrophila auriculata are examined against Staphylococcus aureus, Pseudomonas aeroginosa, Bacillus subtilis, Escherachia coli, Candida albicans and Mycobacterium smegmatis and reported that the leaves exhibited potent anti-microbial activity against Staphylococcus aureus, Bacillus subtilis, Candida albicans and Mycobacterium smegmatis (Boily and Van Puyvelde, 1986) Staphylococcus aureus, *Candida albicans, Mycobacterium canis* and *Trichophyton mentagraphytes*, while the stem exhibited activity against Candida albicans, Mycobacterium canis and Trichophyton mentagraphytes (Vlietinck et al., 1995).

The chloroform and alcoholic extract exhibited significant antibacterial activity, whereas the aqueous extract had moderate activity and the petroleum ether extract had the weakest activity against these microorganisms (Patra et al., 2008). The anthelmintic activity of petroleum ether, chloroform, alcoholic and aqueous extracts of the leaves studied against Pherithima posthuma as a test worm, at different concentrations (10-100 mg/ml) in a bioassay which involved determination of the time until paralysis and time until death of the worms. The alcoholic extract showed significant anthelmintic activity at the highest concentration, 100 mg/ml, whereas chloroform and the aqueous extract were only moderately active and the petroleum ether extract exhibited the weakest anthelmintic activity (Patra et al., 2008). The diuretic potential of the aqueous, alcoholic extract and different fractions of the alcoholic extract of the whole plant of Hygrophila auriculata was examined by treating different groups of Wistar albino rats with single (200 mg/kg) oral doses of alcoholic extract. Furosemide (10 mg/kg) was used as positive control in the study. Of the different fractions, the n-butanol fraction (200 mg/kg) markedly increased the urine output. The pattern of diuresis induced by the nbutanol fraction was almost identical to that produced by furosemide (Hussain and Fareed, 2011). An ethanolic extract of the seeds (100-200mg/kg) administered to rats for the increase in growing frequency (380-472% of control) and similar reductions in growing, intromission, and postejaculatory latencies; all doses were none significantly more libido enhancing (Kanhere et al., 2013) than the active control of 0.5mg/kg testosterone injections.

The aqueous extract of the aerial parts exhibited potent antinociceptive activity in a mouse model of thermally induced analgesia (Shanmugasundaram and Venkataraman, 2006). The petroleum ether, chloroform, alcoholic and aqueous extracts of the leaves were subjected to study analgesic activity by hot plate and tail flick tests in the thermal

method, while the acetic acid-induced writhing test was used in the chemical method. The chloroform, alcoholic and aqueous extracts significantly inhibited the abdominal constriction produced by acetic acid and also increased the pain threshold of mice to the thermal source in a dose-dependent manner comparable with the standard drug, aspirin (Patra et al., 2008).

Molecular studies

A study was conducted to see the HPTLC analysis of TF for the estimation of lupeol. Edema was induced in Wistar albino rats by subplanter injection of 0.1 ml of 1% (w/v) carrageenan into the right hind paw after 1 h of TF administration (100 and 200 mg/kg oral). Septic shock was induced by intraperitoneal administration of LPS (100 µg/kg) in rats and interleukins (IL-1 β and IL-6), tumor necrosis factor (TNF- α), superoxide dismutase (SOD), lipid peroxidation (LPO), and nitric oxide (NO) were measured in serum. AutoDock 4.2 was used for molecular docking. Results showed Administration of TF significantly (p < 0.005) restored the serum levels of cytokines, LPO $(7.77 \pm 0.034 \text{ versus } 4.59 \pm 0.059 \text{ nmole of TBARS})$, NO $(9.72 \pm 0.18 \text{ versus})$ $4.15 \pm 0.23 \,\mu mol$ nitrite/mg of wet tissue), and SOD (4.89 ± 0.036) versus 7.83 ± 0.033 Unit/mg protein) compared with the LPS-challenged rats (Hussain et al., 2016). Analysis of *in silico* results revealed that TNF- α is the most appropriate target in eliciting anti-inflammatory activity. In another study, it was designed to investigate the free radical scavenging activity of the whole plant extract and its fractions. Free radical scavenging activity was assessed by inhibition of 1, 1'-Diphenyl-2-picryl-hydrazyl (DPPH), deoxyribose degradation against OH, nitric oxide and lipid peroxidation radical assays. The TRF of (10–100 µg/ml) exhibited high DPPH radical scavenging capacity. The TRF at the same concentration showed a dose-dependent inhibition of nitric oxide, and hydroxyl radical than AEHA (Hussain et al., 2009). Thus results indicate that the terpenoid-rich fraction from Hygrophila auriculata has the potential to scavenge free radicals and act as an antioxidant.

GC-MS study

The aim of the study was to evaluate the anticancer activity and GC-MS analysis of methanol extract of leaves of H. auriculata. MTT assay method was carried out for anticancer activity on A549 (lung) and HepG2 (liver) cancer cell lines. The maximum cell death of lung cancer cells was 70.54±0.004% and liver cancer cells was 72.37±0.002% at 160 µg/mL concentration of methanol leaves extract of H. auriculata. Antioxidant compound 5-Hydroxy-7-methoxy-2-phenyl-4H-1-Benzopyran-4-one was eluted by GC-MS, which neutralize free radicals, which causes oxidative stress to cells, leads to cancer. The methanol extract of samples were injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Following chromatographic conditions were used: Helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200 °C and column oven temperature was programmed as 50-250 °C at a rate of 10 °C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV; ion source temperature of 250 °C; interface temperature of 250 °C; mass range of 50-600 mass units (Anusha and Immanuel, 2019). The results of GC-MS analysis showed the presence of an antioxidant compound such as 5-hydroxy-7-methoxy-2- phenyl-4H-1benzopyran-4-one in the methanol extract of leaves of H. auriculata, which neutralize

free radicals, which injure tissues. The compound octahydroxanthen-1,9- dione,3,3,7,7- tetramethyl-10-propyl was eluted and the derivative of this compound reported as antibacterial, antiviral and antitumor effects. The compound 8-Amino-5- pchlorophenoxy-6-methoxy-2-methylquinoline was eluted and the derivative of this compound reported as nervous disorder and urinary disorder effects and the compound 2-(pMethoxyphenyl)-4-quinoline carboxamide was eluted, which the derivative was reported as CB1 and CB2 cannabinoid receptors.

Oroxylum indicum

Oroxylum indicum is a species of flowering plant commonly called midnight horror (GRIN, AS, USDA), oroxylum, Indian trumpet flower, broken bones, Indian caper, or tree of Damocles (Figure 4). It can reach a height of 18 metres (59 ft). Various segments of the tree are used in traditional medicine (Harminder and Chaudhary, 2011). O. indicum is a deciduous tree growing throughout India, South Asia, South East Asia, Sri Lanka, Philippines, Indonesia, China, Bhutan, Malaysia and Mallaca. It is found up to an altitude of 1200 m mainly in ravines, in damp region and moist places in the forests (Chauhan, 1999). In India, it is distributed in Himalayan foothills, Eastern and Western Ghats and North East India (Jayaram and Prasad, 2008). It is mostly sighted along the river banks or slopes of the hills. O. indicum lives in relationship with the actinomycete Pseudonocardia oroxyli present in the soil surrounding the roots (Gu et 2006). Septobasidium bogoriense is a fungal species al., responsible for velvet blight in O. indicum (Gu et al., 2006).



Figure 4. Oroxylum indicum with (A) whole tree, (B) leaves, (C) flower, (D) fruits and (E) seeds.

Ethnobotany

The seeds are used in traditional Indian Ayurvedic medicine, included in famous tonic formulations such as *Chyawanprash*. Bark decoction is taken for curing gastric ulcer and a paste made of the bark powder is applied for mouth cancer, scabies and other skin diseases (Chauhan, 1999). *O. indicum* is used as one of the important ingredient in most commonly used Ayurvedic preparations such as *Dasamularistha*,

Syonaka putapaka, Syonaka sidda ghrta, Brhatpancamulyadi kvatha, Amartarista, Dantyadyarista, Narayana Taila, Dhanawantara Ghrita, Brahma Rasayana (Zaveri et al., 2008; Jabbar et al., 2004). The sword like fruit or a branch of the plant is used by the farmers to kill crabs in wet paddy fields. A paste made of the bark is applied to wounds of animals to kill maggots (Warrier et al., 1996). In Thailand, the fruits and flowers of the plant are consumed as a vegetable (Nakahara et al., 2002).

Roots are sweet, astringent, bitter, acrid, refrigerant (Yoganarasimhan and Jadhav, 1996), anti-inflammatory, anodyne, aphrodisiac, expectorant, appetizer, carminative, digestive, anthelmintic, constipating, diaphoretic, diuretic, antiarthritic, antidiabetic and febrifuges. Tonic is useful in dropsy, cough, sprains neuralgia, hiccough, asthma, bronchitis, anorexia, dyspepsia, flatulence, colic, diarrhea, dysentery, gout, vomiting, leucoderma, wounds, rheumatoid arthritis and fever. Root bark is used in stomatitis, nasopharyngeal cancer and tuberculosis (Khare, 2004; Bhattacharje, 2005). Leaves are used as stomachic, carminative and flatulent. Leaf decoction is given in treating rheumatic pain, enlarged spleen (Khare, 2004), ulcer, cough, and bronchitis. Mature Fruits are acrid, sweet, anthelmintic, and stomachic. They are useful in pharyngodynia, cardiac disorders, gastropathy, bronchitis, haemarrhoids, cough, piles, jaundice, dyspepsia, smallpox, leucoderma and cholera (Chauhan, 1999). Seeds are used as purgative. Dried seed powder is used by women to induce conception. Seeds yield nondrying oil used in perfume industry. The seeds are ground with fire soot and the paste is applied to the neck for quick relief of tonsil pain. The medicated oil of O. indicum in sesame oil base instilled into ears mitigates the pain in otitis (GRIN, ARS, USDA). The leaves are very large, 90-180 cm long 2-3 pinnate with 5 or more pairs of primary pinnae, rachis very fast, cylindrical, swollen at the junction of branches, leaflets 2-4 pairs ovate or elliptic, acuminate, glabrous. The flowers are reddish purple outside and pale, pinkish-yellow within, numerous, in large erect racemes. Fruits are flat capsules, 0.33-1 m long and 5-10 cm broad and sword shaped. The pinnate leaves are approximately 1 metre (3.3 ft) in length and comparably wide borne on petioles or stalks up to 2 metres (6.6 ft) in length (Corner, 1952).

Phytochemistry and pharmacology

O. indicum leaves are known to contain flavones and their glycosides, baicalein (5,6,7-trihydroxy flavone) and its 6 and 7-glucuronides, chrysin (5,7-dihydroxy flavone) (Chen et al., 2005; 2003), scutellarein and its 7-glucuronides, anthraquinone and aloeemodin (Jayaram and Prasad, 2008; Dalal and Rai, 2004; Dey, 1978), Ethyl acetate extract of leaves of *O. indicum* contains chrysin (160.9 mg, 97.3% purity), baicalein (130.4 mg, 97.6% purity), baicalein 7-O-glucoside (314.0 mg, 98.3% purity), baicalein-7-O-diglucoside (179.1 mg, 99.2% purity) (Yuan et al., 2008).

From methanol extract of the leaves of *O. indicum*, chrysin-7-O-glucuronide, chrysindiglucoside and baicalein were separated. Structure of the chrysin-diglucoside has yet to be obtained. Stem bark contain flavones oroxylin A (5,7-dihydroxy- 6-methoxy flavone), chrysin, baicalein and its 6 and 7-glucuronide, scutellarin-7-rutinoside, traces of alkaloid (Subramanian and Nair, 1972), tannic acid, sitosterol and galactose, baicalein, biochanin-A, ellagic acid (Dalal and Rai, 2004). Ethyl acetate extract of root of *O. indicum* is reported to contain two flavonoids- i) 2,5-dihydroxy-6,7-dimethoxy flavone and ii) 3,7,3',5',-tetramethoxy-2-hydroxy flavone. Root bark contain chrysin, scutellarin-7-rutinoside, weak acids, traces of alkaloids (Subramanian and Nair, 1972), sitosterol, galactose, baicalein, biochanin-A, ellagic acid, oroxylin-A

and a yellow crystalline coloring matter 5,7-dihydroxy-6-methoxy flavone (Zaveri et al, 2008). The seed oil contains caprylic, lauric, myristic, palmitic, palmotoleic, stearic, oleic and linoleic acids.

Different biochemical activities of O. indicum in various in vivo and in vitro test models. Different part of this plant have been found to exhibit antiinflammatory, antimicrobial, antioxidant, anticancer, antimutagenic, photocytotoxic, antiarthritic, immunostimulant, hepatoprotective, antiproliferative and hepatoprotective activities. Aqueous and ethanol extract of O. indicum were tested for in vitro release of myeloperoxidase (MPO) from rat peritoneal leukocytes. The results indicated that aqueous extract had a significant effect i.e. 64% inhibition of release of MPO (Laupattarakasem et al., 2003). The anti-arthritic activity of different extracts of root bark of Oroxylum indicum was tested against adjuvant - induced arthritis in Male Wistar rats upon inducing the arthritis by injecting 0.1 ml Freund's complete adjuvant intradermally into the left hind paw of the rats (Karnati et al., 2013). The relative percentage inhibition potential of paw volume in rats treated with various extracts of Oroxylum indicum was found to be ethyl acetate extract (67.69%), chloroform extract (64.61%), nbutanol extract (58.46%) respectively. The anti-inflammatory activity was evaluated by carageenan induced rat paw edema model in rats in dosage 50 mg/kg and 300 mg/kg of aqueous extract using diclofenac sodium as standard drug. Result showed that paw volume was significantly reduced in dose dependent manner as compared to control. Dose of 300 mg/kg showed maximum anti-inflammatory activity (Upaganlawar et al., 2009). The active fraction of root bark at a dose level of 100 mg/kg p.o. showed significant reduction (P<0.05) in the ulcer index, total acidity, total acid output, pepsin activity and pepsin output along with a significant rise in total carbohydrate to protein ratio (Khandhar et al., 2006).

The methanolic, ethyl acetate, and ethanolic extracts of stem bark of Oroxylum indicum were tested on three different species of gram positive and gram negative bacteria viz. Bacillus subtilis, E. coli, and Pseudomonas aeruginosa. All of theextractswere found to possess remarkable antibacterial properties. Hexane, CCL4 and chloroform obtained from methanolic stem bark extract shows response against various gram-positive and gram-negative bacteria such as Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus, and Sarcina lutea. E. coli, Pseudomonas aeruginosa, Salmonella parathyphii, Salmonella typhi, Shigella boydii, Shigella dysenteriae, Vibrio mimicus, Saccaromyces cerevaceae, and some fungi such as Candida albicans and Aspergillus niger (Siddiqui et al., 2012). The antioxidant activity of ethanol and aqueous extract of O. indicum leaves was studied in two in vitro models viz. radical scavenging activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) reduction and nitric oxide radical scavenging activity in Griess reagent system. Ethanol extract possessed significant antioxidant activity in both the models. In scavenging DPPH radical, extracts activity was $IC_{50}=24.22 \mu g/ml$ while in scavenging nitric oxide (NO) radical, the activity was $IC_{50}=129.81 \ \mu g/ml$. The result showed that ethanol extract of O. indicum leaves possesses free radical scavenging activity (Upaganlawar et al., 2009). Methanol extract of the fruits of O. indicum inhibited in vitro proliferation of HL-60 cells. The in vitro effects of baicalein on the viability and induction of apoptosis in the HL-60 cell line was further investigated. The results indicated that baicalein has antitumors effects on human cancer cells (Roy et al., 2007). The hypoglycemic activity of extracts of Oroxylum indicum (L) Vent roots has been studied in Wistar albino rats

which shows positive results when applied in particular dosage for a certain period of time in alcoholic and aqueousextracts (Siddiqui et al., 2012).

Molecular studies

A study was conducted to evaluate the effect on CT-26 cell proliferation and migration of 4 flavonoids baicalein, chrysin, oroxylin-A and its glycoside isolated from *Oroxylum indicum*. Data revealed that baicalein exhibited highest inhibitory effect on proliferation and migration on the analyzed tumor cell line. Baicalein at 10 μ M absolutely blocked the proliferation even after 5 days of treatment (Lalou et al., 2013). The results were observed as in vitro anti-furin activity of baicalein as measured against a fluorogenic peptide and pro-hVEGF-C as substrates. Mature VEGF-C is a strong indicator and biomarker of tumor progression. Baicalein being the major constituent of the plant, this data provides scientific rationale for the observed anticancer activity of *O. indicum* and also offered a new lead molecule for future exploration as potential antitumor agents. Another study evaluated the cardioprotective effect of 70% methanolic extract of *O. indicum* Vent.

Root bark against doxorubicin induced cardiomyopathy in female Sprague Dawley rats. Cardiotoxicity was induced by an intra-peritoneal injection of doxorubicin 30 mg/kg body weight for 4 consecutive days after a ten-day pre-treatment of animals with extracts at 200 mg/kg and 400 mg/kg. Drug treatment continued up to the 14th day. Probucol was orally administered at a dose of 20 mg/kg as standard and ECG was recorded. The animals were sacrificed the next day and comparative analysis of serum levels of creatine phosphokinase (CPK), lactate dehydrogenase (LDH), marker Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), tissue antioxidant status based on Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), reduced Glutathione (GSH) and lipid peroxidation (LPO) was carried out. ECG records of extracts treated animals showed comparison depression normal pattern, in to the control with ST and arrhythmia in cardiogram (Menon et al., 2019).

Tissue antioxidant profile (SOD, GSH and GPx) was significantly (p < 0.01) elevated in the cardiac tissue of treated group in dose-dependent manner; lipid peroxidation level was found to decrease with treatment. Comparative analysis of serum markers – CPK, LDH, SGOT and SGPT – among untreated control, standard and extract treated groups revealed that extracts treated at 400 mg/kg dose significantly reduced the levels (p < 0.01). HPTLC fingerprint of extracts revealed 8 bands and detected the presence of chrysin, apigenin and quercetin.

GC-MS study

A comparetive study of compounds present in the chloroform, ethyl acetate and nbutanol fractions of root extract through gas chromatography mass spectroscopy was performed by root of *O. indicum* is used in ayurvedic preparations like Dashmularisht and Chyawanprash. 50 compounds were isolated in chloroform fraction, whereas in ethyl acetate, n-butanol and in aqueous fractions the number of compounds isolated simultaneously were 35, 24 and 22. Two compounds (9, 12-Octadecadienoic acid and baicalein) were common in all fractions. Other important compounds isolated were myristyl myristate, hydroxy methyl furfural, ascorbic acid 6 -hexadecanoate, pseudopelletierine and squalene. First, a total of 42 components were characterized by LC-quadrupole time-of-flight (qTOF)-tandem mass spectrometry (MS/MS), including 23 flavonoid glycosides, 13 flavonoids and six other types of compounds. Then, 17 characteristic components of the 19 common peaks in the chromatographic fingerprints of O. indicum were confirmed. Fifty samples were classified into two groups by hierarchical clustering analysis and orthogonal partial least squares-discriminant analysis, which also identified the 10 main chemical markers responsible for differences between samples. Finally, the quantitative analysis of multiple components with a single marker method was established for simultaneous determination of six main active components in O. indicum by LC-UV with oroxin B was chosen as internal reference substance (Peng et al., 2019). Further, a rapid and efficient method integrating HPLC ionization-qTOF-MS/MS with LC-electrospray analysis was established to comprehensively discriminate and assess the quality of O. indicum samples.

Conclusion

All the four plants selected for this paper were studied far and near for their values in Ayurveda, ethanobotany, and its traditional values overall. Upon the study, it can be concluded that the leaves, roots, root barks, seeds, and fruits and flowers of each of these plants are highly useful in curing locally and tropically a lot of diseases ranging from Rheumatoid arthritis, inflammation to cold and cough, diabetes, can be used as diuretic as well as aphrodisiac agent and in lumbago and other diseases. The seeds of O. indicum are used in making the product "chawanprash" and many other ayurvedic preparations. The pharmacognostical values such as total ash value, water solule and insoluble ash values along fluorescence values, micro and macroscopic values are stated. Phytochemical investigation studies reveals that all the plants contains a lot of phytochemicals such as alkaloids, steroids, flavonoids, tannins, carbohydrates and amino acids. Every plants are pharmacologically important. Every plants were seen to have anti-arthritic and anti-inflammatory, antimicrobial, antioxidant, diurectic and antidiabetic activities, anti-pyretic, and aphrodisiac activities The molecular studies done so far has been studied and discussed in the paper. Alongside all these, the study is aimed to focus on a point where the scientists have a huge opportunity to carry forward the investigations and the do the further research which can actually help the entire world to receive the benefits in upcoming days. The molecular studies reveals the presence of quite a few new phytochemicals and biochemical compounds which shows the way to the scientists for new discoveries in the medical as well as other aspects.

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Conflict of interest

The authors confirm there are no conflict of interest involve with any parties in this research study.

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