



CHEMICAL EVALUATION OF PROXIMATE, VITAMIN AND AMINO ACID PROFILE OF LEAF, STEM BARK AND ROOT OF INDIGOFERA TINCTORIA

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| Article history: | | Abstract: |
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| Received | August, 21 th 2020 | Medicinal plants contain substances with high therapeutic value because they contain multiple bioactive chemicals. Chemical analysis of <i>Indigofera tinctoria</i> leaves, stem bark and roots was evaluated. The result revealed that proximate composition of <i>Indigofera tinctoria</i> leaves contained 12.49 % moisture content (M.C), 87.51 % dry matter (DM), 30.53 % crude protein (CP), 19.02 % crude fibre (CF), 2.44 % ether extract (EE), 13.86 % ash, 36.59 % carbohydrate (CHO) and 254.1 kcal/100g energy (ME). <i>Indigofera tinctoria</i> stem bark contained M.C (6.40 %), DM (93.60 %), CP (5.11 %), CF (54.49 %), EE (2.00 %), ash (10.42 %), CHO (29.98 %) and ME (156.0 kcal/100g). <i>Indigofera tinctoria</i> roots contained MC, DM, CP, CF, EE, ash, CHO and ME at 10.04 %, 89.60 %, 8.22 %, 40.88 %, 1.21 %, 8.43 %, 42.47 % and 210.0 kcal/100g respectively. Vitamin analysis showed that <i>Indigofera tinctoria</i> leaves, stem bark and roots contained β - carotene (8.45, 2.88 and 5.11 mg/100 g), Vitamin B1 (1.94, 0.33 and 1.00 mg/100 g), Vitamin B2 (0.71, 0.21 and 0.50 mg/100 g), Vitamin B3 (0.66, 0.34 and 0.48 mg/100 g), Vitamin B6 (0.32, 0.21 and 0.30 mg/100 g), Vitamin B7 (0.63, 0.01 and 0.16 mg/100 g), Vitamin B9 (0.26, 0.10 and 0.18 mg/100 g), Vitamin B12 (0.21, 0.03 and 0.10 mg/100 g), Vitamin C (14.0, 3.56 and 9.44 mg/100 g), Vitamin D (0.10, 0.01 and 0.06 mg/100 g) and Vitamin K (0.17, 0.07 and 0.12 mg/100 g). Amino acid analysis revealed the presence of threonine, leucine, lysine, valine, tryptophan, glycine, phenylalanine, histidine, methionine, alanine, serine, proline, aspartate, glutamic acid, tyrosine and cysteine in <i>Indigofera tinctoria</i> leaves, stem bark and roots at (7.65 %, 1.22 % and 3.03 %), (5.76, 1.09 % and 2.46 %), (3.11 %, 1.21% and 2.00 %), (7.21 %, 3.53 % and 4.09 %), (1.45%, 0.03% and 1.00 %), (4.76 %, 0.08 % and 2.33 %), (6.33 %, 2.45 % and 3.49 %), (7.42 %, 2.00 % and 3.00 %), (3.49 %, 0.01 % and 2.00 %), (2.41 %, 0.56 % and 1.20 %), (5.23 %, 1.22 % and 1.76 %), (2.87 %, 0.57 % and 1.00 %), (5.32 %, 2.11 % and 3.56 %), (9.66 %, 4.21 % and 5.11 %), (2.45 %, 0.57 % and 1.67 %) and (1.85 %, 0.81 % and 0.89 %) respectively. It was concluded that <i>Indigofera tinctoria</i> leaves, stem bark and roots are loaded with significant quantity of nutrients, vitamins and amino acid (leaves > roots > stem bark). |
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1. INTRODUCTION

Natural plant product remedies are popularly preferred as they are widely available, economically viable, and environmentally friendly with fewer side effects (Upadhyay, 2011; Olafadehan *et al.*, 2020). A wide range of medicinal plant parts are targeted for extraction, including leaves, roots, flowers, fruits, twigs and exudates (Uniyal *et al.*, 2006; Makhosazana, 2015). The extraction solubilizes secondary metabolites in the form of several of such polyphenols as tannins, phenols, terpenoids, flavonoids, saponins and steroids that have complex structures involving many chiral centers that determine their biological activity (Bruneton, 1995; Olafadehan *et al.*, 2020; Alagbe, 2019). Alagbe *et al.* (2020) reported that there are over 250,000 species of medicinal plant out of which WHO (2010) enlisted 21,000 medicinal plant species. Among some of the underexplored medicinal plant is *Indigofera tinctoria*.

Indigofera tinctoria Linn is a leguminous plant belonging to the family fabaceae which is ranked the third largest family of the blossoming plants after Orchidaceae and Asteraceae with approximately 650 genera and 1800 different species (Mohammad et al., 2018; Alagbe and Omokore, 2019). It is wide spread across tropical regions around the world and had been cultivated and highly valued for centuries as the main source of indigo dye, leading to its common name "true indigo". It grows to about 2.5 meters with short, green imparipinnate leaflets, flowers are about 6-10 mm in axillary racemes and characterized by bright red or rosy colour (Shinwari et al., 2006).

Phytochemical screening of *Indigofera tinctoria* reveals the presence of several bioactive chemicals or phytochemicals (tannins, saponins, phenols, flavonoids, terpenoids, alkaloids, steroids etc.), minerals (calcium, phosphorus, potassium, iron, zinc, magnesium, selenium, sodium, copper, manganese, cobalt, molybdenum etc.), vitamins and fatty acids (Yinusa et al., 2007; Bueno et al., 2013; Chakrabarti et al., 2006). Phytochemical constituents performs several pharmacological effects in animals effect such as antibacterial, antifungal, antiviral, anti-inflammatory, hypolipidemic, neuroprotective, anti-allergic, hepato-protective, antipasmodic and antioxidants (Prakash et al., 2007; Shahjahan et al., 2005; Oluwafemi et al., 2020). The plants (leaf, stem bark and roots) have also traditionally been used for the treatment of toothache, abdominal pain, waist pain, piles, epistaxis, rheumatism, stroke and sexually transmitted diseases (Esimon et al., 1999; Abubakar et al., 2006).

In view of these abundant potentials, *Indigofera tinctoria* could be used as an alternative to synthetic antibiotics in animal feed, reduce cost of production and mortality as well as promote food safety and eliminate worrying cases of antibiotic residues in animal products and dangers posed to human health. This experiment was designed to examine the proximate, vitamin and amino acid profile of leaf, stem bark and root of *Indigofera tinctoria*.

2. MATERIALS AND METHODS

2.1 Study Area

The experiment was carried out at Division of Animal Nutrition, Sumitra Research Institute, Gujarat, India during the month of April to June, 2019.

2.2 Sources, collection and preparation of test materials

Fresh leaves, stems and roots of *Indigofera tinctoria* were collected from a local market in Gujarat, India. The plants were authenticated by a plant taxonomist (Dr. Chen Xung). The collected leaves, root and stem bark of *Indigofera tinctoria* were cut separately into pieces, washed with running tap water to remove all dirty particles and oven dried separately at 60°C for 24 hours, it was later removed and grinded into fine powder using mortar and pestle, sieved and stored separately in a labeled air tight container and kept for further analysis.

2.3 Parameters measured

Proximate compositions of the plant samples were determined by using the Official Methods of the Association of Official Analytical Chemist (AOAC, 2000).

Total carbohydrate was determined according the method of James (1995) using the equation below:

$$\text{Total carbohydrate} = 100 - (\% \text{ crude protein} + \% \text{ crude fibre} + \% \text{ ash})$$

$$\text{Dry matter (DM)} = 100 - \text{moisture content}$$

Energy value (kcal/100 g) was determined using the formula described by Crisan and Sands (1978) as follows:

$$\text{Energy (kcal/100 g)} = (2.62 \times \% \text{ crude protein}) + (8.37 \times \% \text{ ether extract}) + (4.2 \times \text{total carbohydrate})$$

Amino acid analyses were carried out using Ion Exchange Chromatographic Method (IECM) model (9B FB- 01F) and other methods outlined by Olafadehan *et al.* (2020).

Vitamin analyses were carried out according to methods outlined by Ngozi *at al.* (2017) and Onwuka (2005).

2.4 Statistical analysis

The analyses were done in triplicates and the data obtained were expressed as mean \pm standard error of the means (mean \pm S.E.M). The data were subjected to one way analysis of variance (ANOVA) and differences between samples were determined Duncan multiple range test (Duncan, 1955). Significant was declared if $P \leq 0.05$.

Table 1 Proximate composition of *Indigofera tinctoria* leaf, stem bark and root

| Parameters | Leaf | Stem bark | Root |
|------------------------|---------------------------|---------------------------|---------------------------|
| Moisture (%) | 12.49 ± 0.36 ^a | 6.40 ± 0.02 ^b | 10.04 ± 0.40 ^a |
| Dry matter | 87.51 ± 0.01 | 93.60 ± 0.17 | 89.60 ± 0.05 |
| Crude protein (%) | 30.53 ± 0.07 ^a | 5.11 ± 0.02 ^c | 8.22 ± 0.01 ^b |
| Crude fibre (%) | 19.02 ± 0.10 ^c | 54.49 ± 0.25 ^a | 40.88 ± 0.59 ^b |
| Ether extract (%) | 2.44 ± 0.00 | 2.00 ± 0.01 | 1.21 ± 0.00 |
| Ash (%) | 13.86 ± 0.08 ^a | 10.42 ± 0.05 ^a | 8.43 ± 0.02 ^b |
| Total carbohydrate (%) | 36.59 ± 0.03 ^a | 29.98 ± 0.01 ^b | 42.47 ± 0.02 ^a |
| Energy (kcal/100g) | 254.1 ± 0.00 | 156.0 ± 0.00 | 210.0 ± 0.00 |

Values expressed as mean ± SEM (n=3)

Means in the same row with different superscripts differ significantly ($P < 0.05$)

Table 2 Vitamin composition of *Indigofera tinctoria* leaf, stem bark and root

| Parameters (mg/100 g) | Leaf | Stem bark | Root |
|------------------------------|--------------------------|--------------------------|--------------------------|
| β- carotene | 8.45 ± 0.44 ^a | 2.88 ± 0.03 ^b | 5.11 ± 0.13 ^a |
| Vitamin B1 (Thiamine) | 1.94 ± 0.00 ^a | 0.33 ± 0.01 ^b | 1.00 ± 0.00 ^a |
| Vitamin B2 (Riboflavin) | 0.71 ± 0.01 | 0.21 ± 0.00 | 0.50 ± 0.01 |
| Vitamin B3 (Niacin) | 0.66 ± 0.02 | 0.34 ± 0.01 | 0.48 ± 0.10 |
| Vitamin B6 (Pyridoxine) | 0.32 ± 0.00 | 0.21 ± 0.01 | 0.30 ± 0.00 |
| Vitamin B7 (Biotin) | 0.63 ± 0.01 ^a | 0.01 ± 0.00 ^c | 0.16 ± 0.01 ^b |
| Vitamin B9 (Folic acid) | 0.26 ± 0.04 ^a | 0.10 ± 0.00 ^b | 0.18 ± 0.00 ^b |
| Vitamin B12 (Cyanocobalamin) | 0.21 ± 0.00 ^a | 0.03 ± 0.01 ^b | 0.10 ± 0.00 ^a |
| Vitamin C (Ascorbic acid) | 14.0 ± 1.22 ^a | 3.56 ± 0.02 ^c | 9.44 ± 1.04 ^b |
| Vitamin D (Calciferol) | 0.10 ± 0.01 | 0.01 ± 0.00 | 0.06 ± 0.01 |
| Vitamin K (Phytonadione) | 0.17 ± 0.00 | 0.07 ± 0.00 | 0.12 ± 0.00 |

Values expressed as mean ± SEM (n=3)

Means in the same row with different superscripts differ significantly ($P < 0.05$)

Table 3 Amino acid composition of *Indigofera tinctoria* leaf, stem bark and root

| Parameters (%) | Leaf | Stem bark | Root |
|--|--------------------------|--------------------------|--------------------------|
| <u>Essential amino acid</u> | | | |
| Threonine | 7.65 ± 0.98 ^a | 1.22 ± 0.01 ^c | 3.03 ± 0.00 ^b |
| Leucine | 5.76 ± 0.02 ^a | 1.09 ± 0.00 ^b | 2.46 ± 0.01 ^a |
| Lysine | 3.11 ± 0.01 | 1.21 ± 0.01 | 2.00 ± 0.00 |
| Valine | 7.21 ± 1.56 ^a | 3.53 ± 0.00 ^c | 4.09 ± 0.02 ^b |
| Tryptophan | 1.45 ± 0.03 | 0.03 ± 0.00 | 1.00 ± 0.00 |
| Glycine | 4.76 ± 0.02 | 0.08 ± 0.00 | 2.33 ± 0.10 |
| Phenylalanine | 6.33 ± 0.01 ^a | 2.45 ± 0.10 ^b | 3.49 ± 0.08 ^b |
| Histidine | 7.42 ± 0.04 ^a | 2.00 ± 0.00 ^b | 3.00 ± 0.02 ^b |
| Methionine | 3.94 ± 0.00 ^a | 0.01 ± 0.00 ^c | 2.00 ± 0.01 ^b |
| <u>Non-essential amino acid</u> | | | |
| Alanine | 2.41 ± 0.10 | 0.56 ± 0.02 | 1.20 ± 0.00 |
| Serine | 5.23 ± 0.14 ^a | 1.22 ± 0.00 ^b | 1.76 ± 0.00 ^b |
| Proline | 2.87 ± 0.02 | 0.57 ± 0.01 | 1.00 ± 0.01 |
| Aspartic acid | 5.32 ± 0.00 ^a | 2.11 ± 0.00 ^b | 3.56 ± 0.03 ^b |
| Glutamic acid | 9.66 ± 1.00 ^a | 4.21 ± 0.01 ^b | 5.11 ± 1.00 ^b |
| Tyrosine | 2.45 ± 0.01 | 0.57 ± 0.00 | 1.67 ± 0.06 |
| Cysteine | 1.85 ± 0.00 | 0.81 ± 0.00 | 0.89 ± 0.03 |

Values expressed as mean ± SEM (n=3)

Means in the same row with different superscripts differ significantly ($P < 0.05$)

3.Result and discussion

3.1 Proximate composition of *Indigofera tinctoria* leaf, stem bark and root

Table 1 reveals the proximate composition of *Indigofera tinctoria* leaf, stem bark and root. *Indigofera tinctoria* leaf, stem bark and root contained moisture (12.49 %, 6.40 % and 10.04 %), dry matter (85.71 %, 93.60 % and 89.60 %), crude protein (30.53 %, 5.11 % and 8.22 %), crude fibre (19.02 %, 54.49 % and 40.88 %), ether extract (2.44 %, 2.00 % and 1.21 %), ash (13.86 %, 10.42 % and 8.43 %), total carbohydrate (30.59 %, 29.98 % and 42.47 %) and energy (254.1, 156.0 and 210.0 kcal/100g) respectively. The moisture content in the sample is lower than the values reported for *Carpolobia lutea* and *Piostigma thonningii* leaf (8.84 % and 7.11 %), root (9.55 % and 8.34 %) respectively by Olayinka et al. (2019) and Alagbe, J.O (2019). Dry matter value obtained in the test material is lower than values for *Desmodium triflorum* (33.46 %), *Clitoria ternatea* (18.61 %), *Stylosanthes guianensis* (27.06 %), *Calapogonium mucunoides* (29.18 %), *Albizia falcate* (35.46 %), *Centrosema pubescens* (33.11 %), *Leucaena leucocephala* (32.59 %), *Indigofera zollingeriana* (25.61 %) and *Arachis pintoi* leaves (30.66 %) but in agreement with the findings of Alagbe et al. (2020) who reported a dry matter of 93.75 % in *Daniellia oliveri* stem bark. Dry matter is an indicator of the quantity of nutrients that are available to the animal in a particular feed or sample. Crude protein (CP), crude fibre (CF) and ash values in sample was higher than those reported for *Eugenia uniflora* leaves (9.58 %, 1.27 % and 9.24 %), stem (6.21 %, 0.67 % and 3.57 %) and root (7.23 %, 0.33 % and 7.00 %) reported by Okoh et al. (2011). Higher CF in stem bark and roots of *Indigofera tinctoria* is an indication that the plant has the ability to lower blood sugar and cholesterol level as well as improving digestion in animals (NHWC, 2002). Leaf of *Indigofera tinctoria* is rich in protein and could be used as a protein supplement in poultry feed (Atteh, 2000; Alagbe, 2019). The ether extract value obtained in this experiment is lower than those reported for *Moringa olifera* leaves (0.40 %), stem bark (0.15 %) and roots (2.28 %) respectively reported by Olorunfemi et al. (2013). Ash content is a parameter used to access the mineral content in a sample (Onwuka, 2005; Alagbe, 2019). The result revealed that *Indigofera tinctoria* leaf contained appreciable amount of minerals followed by its stem bark and root respectively. Minerals are spark plug of life necessary for enzyme activation in the body (Gupta et al., 2014). The presence of minerals in livestock feed is important for the animals metabolic processes and its disturbance could lead to deficiencies or variety of diseases (Soetan et al., 2010). According to Aiyesanmi and Oguntokun (1996) fats are essential in diets for energy, structural and biological functioning of cells in the body. Total carbohydrates (CHO) and energy analysis result showed that the leaves of *Indigofera tinctoria* > roots > stem bark. The value obtained is lower than the values reported for *Mangifera indica* leaves 60.61% (CHO); 346.14 (kcal/100g), *Persea americana* leaves 66.04% (CHO); 370.47 (kcal/100g) and *Annona muricata* leaves 65.56 % (CHO); 352.02 (kcal/ 100g) as reported by Princewill et al. (2019). The result revealed that the test material is lower in energy and thus, it cannot be used as an energy source in livestock feed.

3.2 Vitamin composition of *Indigofera tinctoria* leaf, stem bark and root

Table 2 reveals the vitamin composition of *Indigofera tinctoria* leaf, stem bark and root. The sample contained β – carotene at 8.45 mg/100g, 2.88 mg/100g and 5.11 mg/100g, vitamin B1 (1.94 mg/100g, 0.33 mg/100g and 1.00 mg/100g), vitamin B2 (0.71 mg/100g, 0.21 mg/100g and 0.50 mg/100g), vitamin B3 (0.66 mg/100g, 0.34 mg/100g and 0.48 mg/100g), vitamin B6 (0.32 mg/100g, 0.21 mg/100g and 0.30 mg/100g, vitamin B7 (0.63 mg/100g, 0.01 mg/100g and 0.16 mg/100g), vitamin B9 (0.26 mg/100g, 0.10 mg/100g and 0.18 mg/100g), vitamin B12 (0.21 mg/100g, 0.03 mg/100g and 0.10 mg/100g), vitamin C (14.0 mg/100g, 3.56 mg/100g and 9.44 mg/100g), vitamin D (0.10 mg/100g, 0.01 mg/100g and 0.06 mg/100g) and vitamin K (0.17 mg/100g, 0.07 mg/100g and 0.12 mg/100g) for *Indigofera tinctoria* leaf, stem bark and root respectively. The result revealed that *Indigofera tinctoria* leaf contained significant quantity of vitamins followed by roots and stem bark. Vitamins are chemically complex organic compounds that have significant role in growth and development of the human body (Muhammad et al., 2017). Vitamin A, E, K and D are fat soluble vitamins whereas, vitamin B1, B2, B3, B6, B7, B9, B12, biotin and vitamin C are water soluble vitamins. β – carotene are precursors of vitamin A and it plays a key role in good sight or vision as well as cell growth and development (Shearer et al., 2012, 2014; Mata-Granados et al., 2010). According to Jolliffe et al. (2013), vitamin D is important for normal body functioning as its deficiency cause the malformation and softening of bones. Vitamin E protects the membrane fats from oxidative damage and maintains the cellular functioning of the body (Muhammad et al., 2017). Vitamin K plays a vital role photosynthesis, antioxidants and energy generation by electron movement (Kurosu and Begari, 2010). Vitamin B1 is responsible for the proper functioning of the brain, digestive system, heart and central nervous system (Ba, 2008). Vitamin B2 promotes iron metabolism for the production of red blood cell (Lanska, 2010). According to Combs (2007), vitamin B3 has the ability to scavenge free radicals and protect the tissues from oxidative damage. Vitamin B6 is very important vitamin as it is involved in red blood cell production, carbohydrate metabolism, liver detoxification, brain and nervous system health (Combs, 2007; WHFoods, 2017). Biotin plays a key role in sugar and fat metabolism (WHFoods, 2017). Folic acid (Vitamin B9) regulates the homocysteine level in blood and this amino acid is marker for cardiovascular diseases (McDowell, 2012; Crider et al., 2011). Vitamin B12 vitamin also plays important role in energy metabolism and other biological

processes (Asensi et al., 2010; Hayden and Tyagi, 2004). Vitamin C is a strong antioxidant giving full protection to the body and also capable of protecting the lens of eyes, molecules circulating in bloodstream genetic material (DNA) from harmful effects of free radicals (Youdim et al., 2000; Mata-Granados et al., 2010). Vitamin D is essential in calcium absorption in the blood (Fauschou et al., 2012; Jolliffe et al., 2013; Belenchia et al., 2013). Vitamin E exerts positive effect on fertility and absorption of iron in the blood (Marshall, 1986; Zhao et al., 2014). Vitamin K is very essential for its help in blood clotting (Kurosu and Begari, 2010; Alagbe et al., 2020).

3.3 Amino acid composition of *Indigofera tinctoria* leaf, stem bark and root

Table 3 reveals the amino acid composition of *Indigofera tinctoria* leaf, stem bark and root. The test material contained threonine (7.65 %, 1.22 % and 3.03 %), leucine (5.76 %, 1.09 % and 2.46 %), lysine (3.11 %, 1.21 % and 2.00 %), valine (7.21 %, 3.53 % and 4.09 %), tryptophan (1.45 %, 0.03 % and 1.00 %), glycine (4.76 %, 0.08 % and 2.33 %), phenylalanine (6.33 %, 2.45 % and 3.49 %), histidine (7.42 %, 2.00 % and 3.00 %), methionine (3.94 %, 0.01 % and 2.00 %), alanine (2.41 %, 0.56 % and 1.20 %), serine (5.23 %, 1.22 % and 1.76 %), aspartic acid (5.32 %, 2.11 % and 3.56 %), glutamic acid (9.66 %, 4.21 % and 5.11 %), tyrosine (2.45 %, 0.57 % and 1.67 %) and cysteine (1.85 %, 0.81 % and 0.89 %) for *Indigofera tinctoria* leaf, stem bark and root respectively. The result obtained in this study is lower than values reported for *Moringa oleifera* and *Psidium guajava* leaves, stem and roots by Olorunfemi et al. (2013); Essiet et al. (2016) respectively. Amino acid screening revealed that all the parameters follow similar trend (*Indigofera tinctoria* leaves > stem bark > roots). Amino acids (AA) were traditionally classified as nutritionally essential or nonessential for animal (Guoyao, 2010).

Glycine is necessary for central nervous system and healthy prostate (Mohammad et al., 2017; Wu et al., 2004). Lysine plays a key role in cell division and growth; its deficiency can lead to impaired fatty acid metabolism and defective connective tissues (Elango et al., 2009). Methionine is essential to cartilage and liver health (Chen et al., 2007; Pali et al., 2009). Isoleucine is vital for the regulation of blood sugar and aids to increase the rate of protein synthesis and muscle tissue formation (Elango et al., 2009; Chmurzynska, 2010). Phenylalanine is necessary in glucose production for the brain, insulin secretion and fat oxidation (Brosnan et al., 2010). Threonine is paramount in muscle tissue production and also contributes to the neurotransmitter balance in the brain (Watford, 2008). Alanine and glutamic acid protects the cardiovascular system as well as supply energy to the cells of the body (Bruhat et al., 2009). Arginine assists to ensure a healthy immune system and release of growth hormones and insulin in human being (Wu et al., 2004; Tan et al., 2009). Aspartic acid is an excitatory brainstem and spinal cord neurotransmitter that increases the chance of postsynaptic membrane depolarization (Deng et al., 2009). Proline and Serine are necessary in collagen synthesis and epinephrine, creatinine, DNA, RNA production (Brosnan et al., 2010; Brasse et al., 2009). Tyrosine serves as a building block for polypeptide and protein synthesis (Wang et al., 2008).

4. Conclusion

Medicinal plants are a reservoir of biologically active compounds with therapeutic properties. This is because plant phytochemicals or bioactive chemicals (tannins, phenols, terpenoids, flavonoids, saponins, steroids etc.) possess enormous scaffolds that are mimicked in the design of most molecular structured synthetic drugs or even modified further to enhance a drug's biological activity profile. Thus, there has been a renewed interest in investigating natural products because they are relatively cheap, safe and effective.

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