

POLYPHENOLS AND FLAVONOIDS OF TWELVE INDIAN MEDICINAL PLANTS

SUMITRA CHANDA*, DILIP BHAYANI AND DISHANT DESAI

Phytochemical, Pharmacological and Microbiological Laboratory,
Department of Biosciences, Saurashtra University, Rajkot - 360 005, Gujarat, INDIA
e-mail: svchanda@gmail.com

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*Corresponding
author

ABSTRACT

In the present work, twelve Indian medicinal plants (*Averrhoa carambola* L., *Buchanania lanzan* Spr., *Calophyllum inophyllum* L., *Celastrus paniculatus* Willd., *Clerodendron multiflorum* L., *Luffa acutangula* (L.) Roxb., *Morinda citrifolia* L., *Ocimum gratissimum* L., *Peltophorum ferrugineum* (Decne.), *Phyllanthus fraternus* Webster Syn., *Triumfetta rotundifolia* Lam. and *Ziziphus nummularia* (Burm. F.) were evaluated for their total phenol and flavonoid content. The plant material was extracted individually in different solvents by cold percolation method. Total phenol and flavonoid content was measured by using Folin-Ciocalteu's reagent method and aluminum chloride colorimeter method respectively. Amongst the twelve plants screened, methanolic extract of *P. ferrugineum* had highest total phenol and flavonoid content. These results suggest that the methanolic extract of *P. ferrugineum* can be considered as a medicinal source for the treatment and prevention of many free radical related diseases.

INTRODUCTION

The use of traditional medicine is widespread and plants still present a large source of novel active biological compounds with different activities, including anti-inflammatory, anticancer antiviral, antibacterial and cardioprotective activities. Antioxidants may play a role in these health promoting activities (Yan *et al.*, 2002). Natural products which contain antioxidant properties such as phenolics, include flavonoids and phenolic acids (Klimczak *et al.*, 2007), carotenoids and vitamins (Rupasinghe and Clegg, 2007).

Scientific studies of plants used in ethno medicine led to the discovery of many valuable drugs, including taxol, comptothecin, vincristine and vinblastine (Gupta *et al.*, 2004). The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites that possess an aromatic ring bearing one or more hydroxyl constituents. Current interest in them stems from their antioxidants, anti-inflammatory, anti-mutagenic and anticarcinogenic activities (Thompson, 2000; Atoui, *et al.*, 2005).

Phenolics are antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Pietta, 2000). They also have metal chelation properties (Kahkonen *et al.*, 1999), their significance for the human diet and antimicrobial activity has been established (Nychas *et al.*, 2003). The antioxidant properties of these compounds are often claimed for the protective effects of plant-based beverages against cardiovascular disease, certain forms of cancer and photosensitive reactions (Haslam, 1998; Bravo, 1998). It was also found that they inhibit human immunodeficiency viral replication (HIV), human simplex virus

(HSV), glucosyl transferases of streptococcus mutans (dental carries), ascorbate auto oxidation (green tea), cytotoxic effects, tumor promotion and xanthine monoamine oxidases (Havsteen, 2000; Mattila *et al.*, 2000). These studies provide the basis for the present rapidly increasing interest for the use of natural antioxidant as functional food ingredients or as food supplements.

Plants containing flavonoids have been reported to possess antioxidant properties (Raj and Shalini, 1999). The antioxidant properties of flavonoids are due to several different mechanism, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free radical generation (Acker *et al.*, 1996; Benavente - Garcia *et al.*, 1997). There is evidence that flavonoids have antiphosphodiesterase activity and could thus elevate intracellular levels of cyclic nucleotides (Abdollahi *et al.*, 2003). Recent studies indicate the both cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) can diminish oxidative stress in many biological system and diseases *in vivo* (Abdi *et al.*, 2005; Milani *et al.*, 2005).

The medicinal value of the plants lies in some chemical substances that produce a definite physiological action on the human body. These effects have been attributed to the peptides, alkaloids, essential oils, phenols and flavonols which are major components of the plants (Okigbo and Igwe, 2007). Hence in the present work, twelve plants have been selected which are traditionally used to treat various diseases for evaluating their total phenol and flavonoid content.

The selected plants are *Averrhoa carambola* L., *Buchanania*

lanzan Spr., *Calophyllum inophyllum* L., *Celastrus paniculatus* Willd., *Clerodendron multiflorum* L., *Luffa acutangula* (L.) Roxb., *Morinda citrifolia* L., *Ocimum gratissimum* L., *Peltophorum ferrugineum* (Decne.) , *Phyllanthus fraternus* Webster Syn., *Triumfetta rotundifolia* Lam. And *Ziziphus nummularia* (Burm.F.) and various medicinal importances of these plants are reported in literature.

Description of twelve selected plants

***Averrhoa carambola* L.**

Averrhoa carambola L. belongs to the family Oxalidaceae. Its vernacular name is Kamarakha. It is a small tree with close drooping branches. It is cultivated in India. It is introduced by Portuguese. The leaves are alternate, entire, and glabrous. The Inflorescence is short racemes. In the flower, the petals are glabrous; the sepals are oblong-obovate. The fruits are oblong, acutely long angled yellow. The ripe fruit is sour and highly cooling. It is used as a laxative, refrigerant and anti-scorbutic; Fruit syrup is used in internal piles, fruit is digestible, stops diarrhea, vomiting and allays thirst, used as a blood purifier. The leaves are used in scabies, antipruritic and antipyretic.

***Buchanania lanzan* Spr.**

Buchanania lanzan Spr. belongs to the family Anacardiaceae. Its vernacular name is charoli. It is found throughout India in deciduous forest, more common in Eastern and Southern India. It is an evergreen tree with a straight cylindrical trunk up to 15cm height. The branches are tomentose when young, bark rough. The leaves are thickly coriaceous, broadly oblong, obtuse, base rounded. The inflorescence is axillary and terminal panicles. The flowers are small, greenish white. The fruits are drupes ovoid or globoid, stones hard. The fruit is laxative, cooling, aphrodisiac, cures biliousness, fever, thirst, ulcer and blood diseases. The paste of young trees mixed with CaCO₃ is applied externally in snake bite.

***Calophyllum inophyllum* L.**

Calophyllum inophyllum L. belongs to the family Guttiferae. Its vernacular name is nagchampo. It is found in humid tropics. It is a small handsome tree, bark grey, smooth. The leaves are, broadly elliptic, rounded at the apex, at times emarginated, margins slightly wavy, nerves compact, prominent. The inflorescence is lax, few flower raceme, 10-15cm long. The flowers are 1.9-2.5cm in diameter, white fragrant, sepals 4, ovate orbicular, concave, reflexed, ciliolate, petals-4, oblong-obtuse, spreading the stamens are numerous, filaments united into 4-6 bundles style: long, twisted, stigma large, mushroom like. The fruit is globose, small, smooth and yellowish. The Oil is purgative and emetic; bark is astringent. Bark is used in ulcers, inflammations of the eyes, constipation. Oil is used in rheumatism, urinogenital irritation of gonorrhoea and gleet. Seed decoction is used in dressing ulcers and wounds, crushed in oil and applied on painful joints. Commercially theoil is a medium of choice for preparing cosmetics and soaps. Oil cakes are used as manure.

***Celastrus paniculatus* Willd.**

Celastrus paniculatus Willd. Belongs to the family Celastraceae. Its vernacular name is Malkangana. It is found in South Gujarat, Central India, Madras, Sri Lanka, Burma and Malaya. It is large, extensive, deciduous twiners, with pale-

brown, watery- lenticellate bark. The leaves are 3.5-17 × 2-12 cm, alternate, entire, broadly ovate, obovate, glabrous, Inflorescence terminal and axillary racemes; 7-20 cm. The flowers are greenish-yellow, 3.8 mm diameter. The fruit is 0.4-0.8 cm diameter, capsule, bright-yellow, deep red or orange when ripe, subglobose or ovoid. The stem bark is abortifacient, the seed is bitter, laxative, emetic, alterative, stimulant, aphrodisiac; Oil is stimulant, rubefacient, stomachic and tonic. The leaf is emmenagogue, used in beriberi, enriching blood, abdominal complaints, cough, asthma, headache, leucoderma.

***Clerodendron multiflorum* L.**

Clerodendron multiflorum L. belongs to the family Verbenaceae. Its vernacular name is Ami. It is found throughout Baluchistan, Sri Lanka and India. It is 2.5-4m, large bushy shrub; the bark is creamy white to grey. The leaves are 0.6-0.3 × 0.3-6.5 cm, cauline opposite, exstipulate, simple, broadly ovate, thinly hairy. The inflorescence is axillary dichotomous, cymes and terminal panicles. The flowers are creamy white, sometimes with pinkish tinge, bracteolate complete, actinomorphic, pentamerous, fragrant. The calyx is gamosepalous, valvate, glabrous, persistent segments ovate, acutely acuminate, veined. The corolla is gamopetalous, hypocrateriform, tube 2-2.5cm, glabrous inside, lobes elliptic and obtuse, veined. The fruits are Drupes 0.4-0.6 cm diameter, obovoid, glabrous or sparsely hairy, depressed, 4 lobes with 1 pyrene in each lobe. The root is bitter, tonic, demulcent.

***Luffa acutangula* (L.) Roxb.**

Luffa acutangula (L) Roxb. belongs to the family Cucurbitaceae. Its vernacular name is Turiya. Its english name ribbed gourd. It is cultivated throughout India. It is an extensive annual climber. Stem 5 angular, glabrous often with scabrous angles. Leaves are broadly ovate to roundish, 5-7 angled or lobed, glabrous, petioles 8-12cm long, scabrous. Inflorescence is axillary racemes. In flowers, Male flowers on 10-15cm long peduncles and female flowers on 5-10cm long peduncles, 15-20 flowers, pedicels 1-4cm long, bract is ovate 3-7 mm long, fleshy green with 3-10 glistening glands on upper surface, Calyx Tube campanulate, teeth lanceolate, winged. Petals are bright yellow in colour and obcordate. Fruits are 10 angled constricted at apex. Fruits are used as demulcent, diuretic, tonic, nutritive. Seeds are bitter, emetic, expectorant, purgative and used in dermatopathy. Leaves are used in splenitis, hemorrhoids, leprosy, granular conjunctivitis, ring worm.

***Morinda citrifolia* Linn.**

Morinda citrifolia Linn. belongs to the family Rubiaceae. Its vernacular name is Noni. A small glabrous tree, trunk straight, bark smooth yellow-white, branch-acute, acuminate or obtuse, bright green, glabrous, shining, petioles ½ inch long, stipule connate, short, broad obtuse, membranous flowera white, in dense ovoid heads over 1 inch long, peduncles solitary, usually leaf opposed, 1-2 inch long, calyx-limb truncate, corolla infundibuliform, tube 3/8 inch long, the mouth hairy, lobes 5, lanceolate, acute, stamens 5, filaments hairy, anthers about ½ exerted, fruit white when ripe. Med.uses: Coughs, nausea and colic, possibly due to its anti-inflammatory activity.

***Ocimum gratissimum* Linn.**

Ocimum gratissimum Linn. belongs to the family Lamiaceae.

Its vernacular name is Jangali tulsi. Shrubby perennial, 4-6 ft. high, much branched, woody below, stem and branches sub quadrangular, the young ones pubescent leaves $2\frac{1}{2}$ 1/2111 - 5 by $1\frac{1}{2}$ - $2\frac{1}{2}$ 1/2111 inch, elliptic- lanceolate, acute coarsely crenate-serrate, pubescent on both sides, gland dotted, base cuneate, petioles 1- $2\frac{1}{2}$ 1/2111 inch long, flowers in simple or branched rather short racemes, in tolerably close whorls, rachis quadrangular, bracts sessile longer than the calyx, acuminate from a broad ovate base, pedicels shorter than the calyx, 1/8 inch long in flower corolla 1/6 inch long pale greenish yellow color, stamens exserted, upper filaments with a bearded tooth at the base nutlets 1/16 inch in diameter. Med.uses: Cephalgia, colic, rheumatoid arthritis, leucoderma, diarrhea.

***Peltophorum ferrugineum* (Decne.)**

Peltophorum ferrugineum (Decne.) Benth. belongs to the family Papilionaceae. Its vernacular name is Paltopham. It is a tree, cultivated in gardens. The leaf-lets are 6-17 pairs. The leaves are edible, emetic and used in diarrhea.

***Phyllanthus fraternus* Webster Syn.**

Phyllanthus fraternus Webster Syn. belongs to the family Euphorbiaceae. Its vernacular name is Bhonya ambli. It is distributed throughout India, Ceylon, Tropics. Tropics generally except Australia. It is an annual, erect herb. The leaves are alternate, numerous, elliptic- oblong or obovate, glabrous, base rounded; petioles very short. The inflorescence is axillary in males and solitary in females. Flowers are Pale-greenish- yellow, minute and numerous. Fruit are bitter, Capsule type, depressed globose, smooth, and scarcely lobed. The plant is good for sores, in chronic dysentery; it's used as diuretic in dropsical affections, gonorrhoea and other troubles of genito-urinal tract. Infusion of the young shoot is given in dysentery. The powdered roots and leaves are made into poultice with rice water to lessen edematous, swellings and ulcers. The decoction of roots and leaves is given in intermittent fevers.

***Triumfetta rotundifolia* Lam.**

Triumfetta rotundifolia Lam. belongs to the family Tilliaceae. Its vernacular name is Jipto. Leaves are Round or orbicular, base is cuneate, margin is toothed, and surface is glabrous. Inflorescence is Raceme. Flowering month is August to December. Flower Shape is ovoid.in flower, Calyx linear and apiculate, Corolla obovate and Stamen 15 to 25 in no. This plant branches used as broom in the villages, used in gonorrhoea. They are mucilaginous and astringent.

***Ziziphus nummularia* (Burm.F.)**

Ziziphus nummularia (Burm.F.) Wight and Arn. Belongs to the family Rhamnaceae. Its vernacular name is Moti bordi. It is found in North-west India and Andhra Pradesh. The leaves: 1- 2.5cm, ovate, elliptical or oval. Leaf surface is pubescent. The stem is spiny, erect or strong stem and branches are in Zigzag manner. The fruit is a drupe and oblong in shape. Fruits are edible and bark contains tannin.

MATERIALS AND METHODS

Collection of plant material: The plants or plant parts were collected from Gir forest of Saurashtra region and Rajkot, Gujarat, India. All the plants were identified by Dr. N. K. Thakrar at Department of Biosciences, Saurashtra University, Rajkot.

The plants were thoroughly washed with tap water, shade dried, crushed in a homogenizer to fine powder and stored for further studies.

Extraction

The plants were extracted by cold percolation method (Parekh and Chanda, 2007) individually using different organic solvents like Petroleum ether, acetone and methanol. 10g of dried powder was taken in 100 ml of Petroleum ether in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24h. After 24h, the extract was filtered with eight layers of muslin cloth; centrifuged at 5000 rpm for 10 min. The supernatant was collected and the solvent was evaporated. The residue was then taken in 100 ml of solvent (acetone and methanol) in a conical flask. Then the procedure followed same as above and the dry extract was stored at 4°C in air tight bottles. The residues were weighed to obtain the extractive yield.

Determination of total phenol content

Total phenol content of the organic solvent extracts of different plants was determined with Folin-Ciocalteu reagent method (Mc donald *et al.*, 2001). 0.5mL sample (1mg/mL in distilled water) was taken in triplicates and 0.1mL Folin-Ciocalteu reagent (0.5 N) was mixed and then incubated for 15 minutes at room temperature. Then 2.5mL of saturated sodium carbonate was added and mixture was again incubated for 30 minutes at room temperature. The absorbance of the mixture was measured at 760nm. Gallic acid was used as a standard. Total phenol values are expressed in terms of gallic acid equivalent (mg g⁻¹ of extracted compound).

Determination of flavonoid content

The flavonoid content of the extracts of different plants was determined with aluminium chloride colorimetric method (Chang *et al.*, 2002). 1.0mL sample (1 mg mL⁻¹ in methanol) was taken in triplicates, 1.0mL methanol, 0.5mL 1.2% aluminium chloride and 0.5mL 120 mM potassium acetate were mixed and incubated for 30 minutes at room temperature. The absorbance of all the samples was measured at 415nm. Quercetin was used as standard. Flavonoid content is expressed in terms of quercetin equivalent (mg g⁻¹ of extracted compound).

RESULTS AND DISCUSSION

Extractive yield

The extractive yield of all the twelve plants in three solvents is given in Fig.1. There was no significant difference in yield of the acetone and petroleum ether extracts but it was less than that of methanol extracts. The higher yield of the methanol extracts suggests higher proportion of methanol soluble plant components. In all the plants, the extractive yield was maximum in methanol and minimum in petroleum ether. Maximum methanolic yield was in *P. ferrugineum* and minimum in *T. rotandifolia* (Fig.1C). Maximum petroleum ether yield was in *P. fraternus* and minimum in *L. acutangula* (Fig.1B, 1C). The extraction yields with different solvents varied from 15.32% to 0.70% per 100 g dry powder (Fig.1) and can be ranked from high to low in the following order: methanol extract > acetone extract > petroleum ether extract.

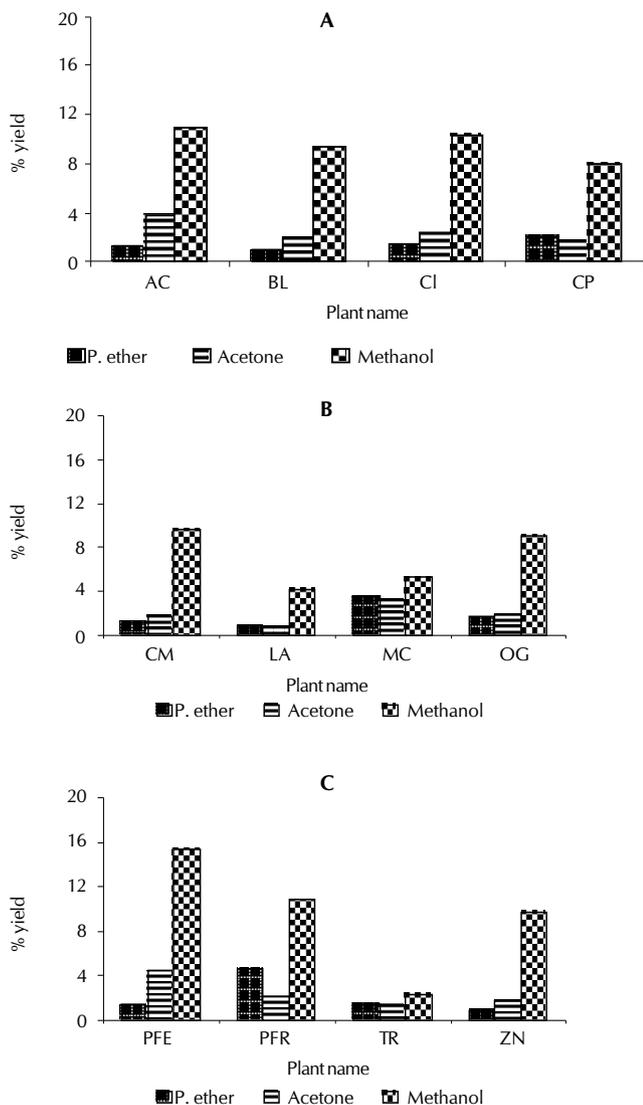


Figure 1: Extractive yield of different plant extracts

AC = *A. carambola*, BL = *B. lanzan*, CI = *C. inophyllum*, CP = *C. peniculatus*, CM = *C. multiflorum*, LA = *L. acutangula*, MC = *M. citrifolia*, OG = *O. gratissimum*, PFE = *P. ferrugineum*, PFR = *P. fratremus*, TR = *T. rotandifolia*, ZN = *Z. nummularia*

Extraction is critical to the recovery of antioxidants and phytochemicals. The extraction yields depend on solvent, time and temperature of extraction as well as the chemical nature of the sample. Under the same time and temperature conditions, the solvent used and the chemical property of sample are the two most important factors (Shimada *et al.*, 1992). There are many reports in the literature where different solvents gave different extractive yield (Parekh *et al.*, 2005; Vaghasiya and Chanda, 2007; Ncube *et al.*, 2008).

Total phenol-flavonoid content

Phenolics can be classified into two groups, polyphenols and simple phenols which contain phenolic acids (Marinova *et al.*, 2005). Plant phenolics are commonly found in both edible and non-edible plants and have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolics is mainly due to their redox

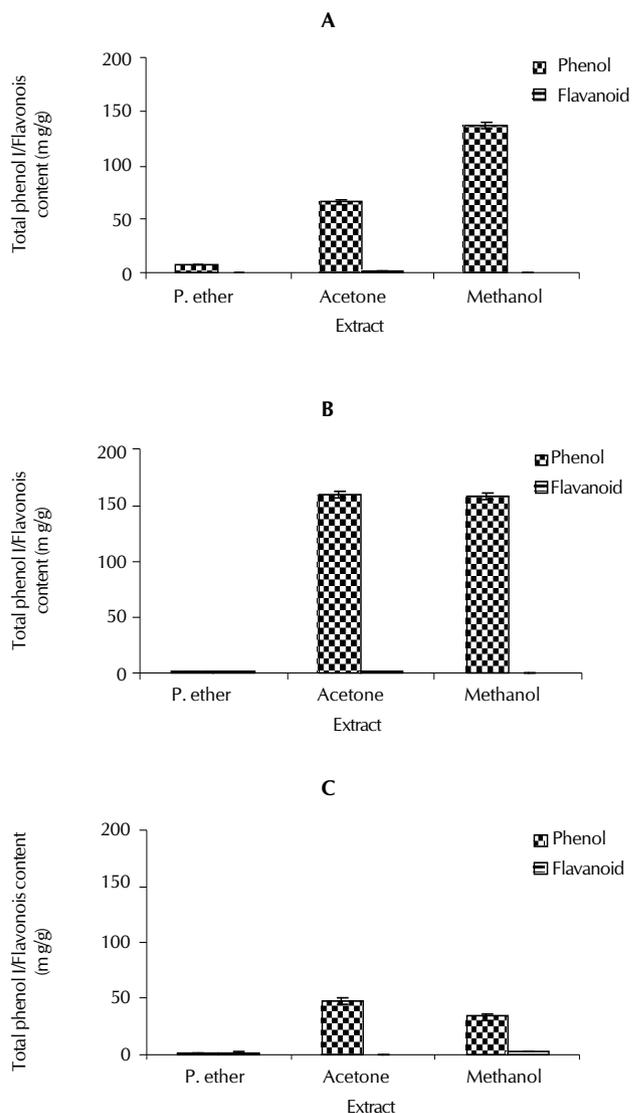


Figure 2: Total phenol and flavonoid content of different solvent extracts of *A. carambola*, *B. lanzan*, *B* and *C. inophyllum*, *C*.

properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. In addition, they have a metal chelation potential (Rice-Evans *et al.*, 1995). The phenolic compounds are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Kahkonen *et al.*, 1999). The majority of free phenolics are flavonols, whereas bound phenolics are mainly phenolic acids (Holtekjolen *et al.*, 2006).

Flavonoids are a large group of naturally occurring plant-phenolic compounds including flavonones and chalcones. Many studies have suggested that flavonoids exhibit numerous biological activities, such as anti-allergenic, antiviral, anti-inflammatory, hepatoprotective, antioxidant, antithrombotic and anticarcinogenic activities (Ameha *et al.*, 2006). Flavonoids like many other polyphenols are excellent free radical scavengers because they are highly reactive as hydrogen or electron donors (Amic *et al.*, 2003; Soobrattee *et*

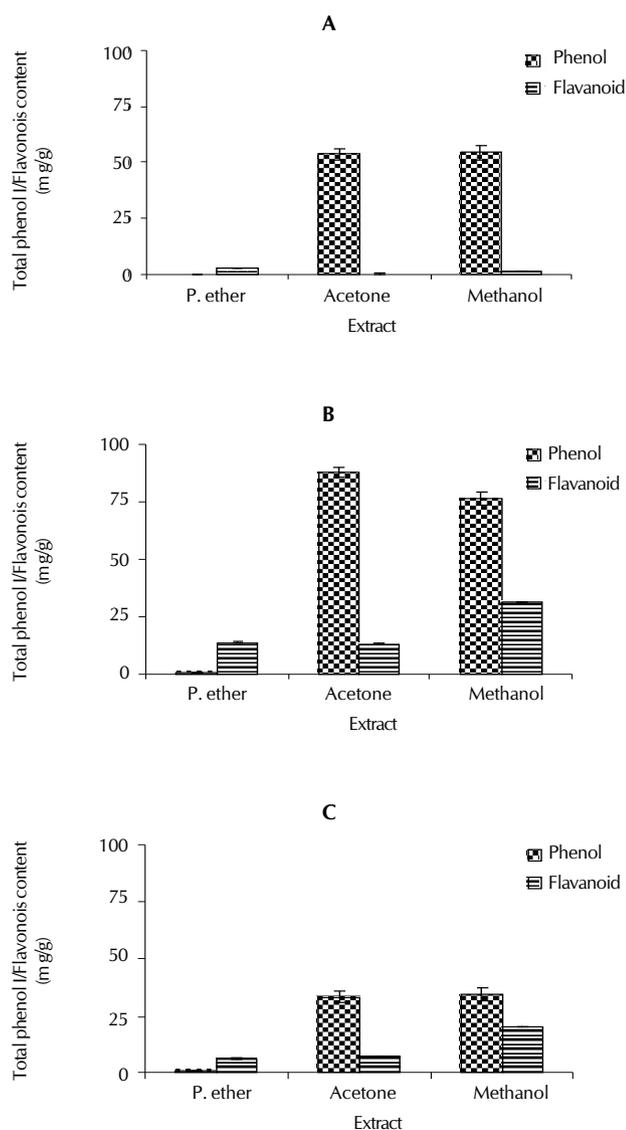


Figure 3: Total phenol and flavonoid content of different solvent extracts of *C. paniculatus* A, *C. multiflorum* B and *L. acutangula* C.

al., 2005). Total phenol and flavonoid content of different solvent extracts of screened plants is given in Figs. 2 - 5.

In different extracts of *A. carambola*, methanol extract showed maximum total phenol content and petroleum ether extract showed minimum total phenol content (Fig. 2A). In different extracts of *B. lanzan* and *C. inophyllum*, acetone extract showed maximum total phenol content, while petroleum ether extract showed minimum total phenol content (Fig. 2B and Fig. 2C).

In different extracts of *C. paniculatus* and *L. acutangula*, total phenol content was highest in methanolic extract followed by acetone extract while it was very less in petroleum ether extract (Fig. 3A and Fig. 3C). Flavonoid content was very much less in all the three extracts of *C. paniculatus* (Fig. 3A) and Flavonoid content was more in methanolic extract and minimum in acetone extract followed by petroleum ether extract of *L. acutangula* (Fig. 3C). In different extracts of *C. multiflorum*

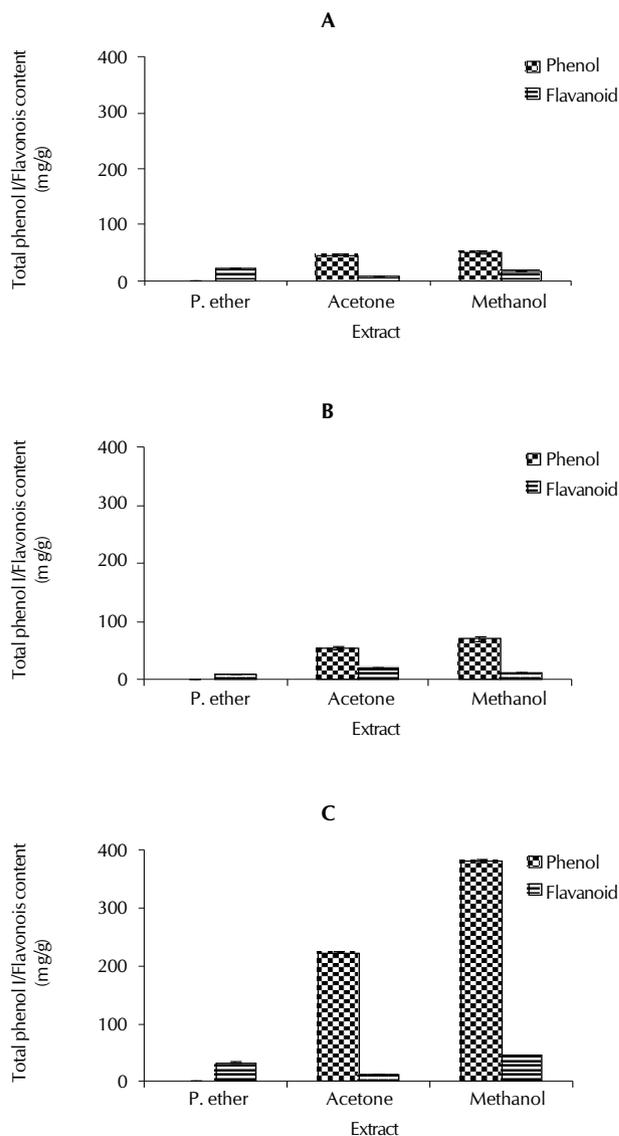


Figure 4: Total phenol and flavonoid content of different solvent extracts of *M. citrifolia* A, *O. gratissimum* B and *P. ferrugineum* C.

total phenol content was maximum in acetone extract and minimum in petroleum ether extract. Flavonoid content was maximum in methanolic extract and minimum in acetone extract followed by petroleum ether extract (Fig. 3B).

In different extracts of *M. citrifolia*, total phenol and flavonoid content was maximum in methanolic extract and minimum in petroleum ether extract (Fig. 4A). In different extracts of *O. gratissimum*, total phenol content was maximum in methanolic extract and minimum in petroleum ether extract. Flavonoid content was maximum in acetone extract and minimum in petroleum ether extract (Fig. 4B). In different extracts of *P. ferrugineum*, total phenol content was maximum in methanolic extract and minimum in petroleum ether extract. Flavonoid content was maximum in methanolic extract and minimum in acetone extract (Fig. 4C).

In different extracts of *P. fraternus*, total phenol content was maximum in methanolic extract and minimum in petroleum

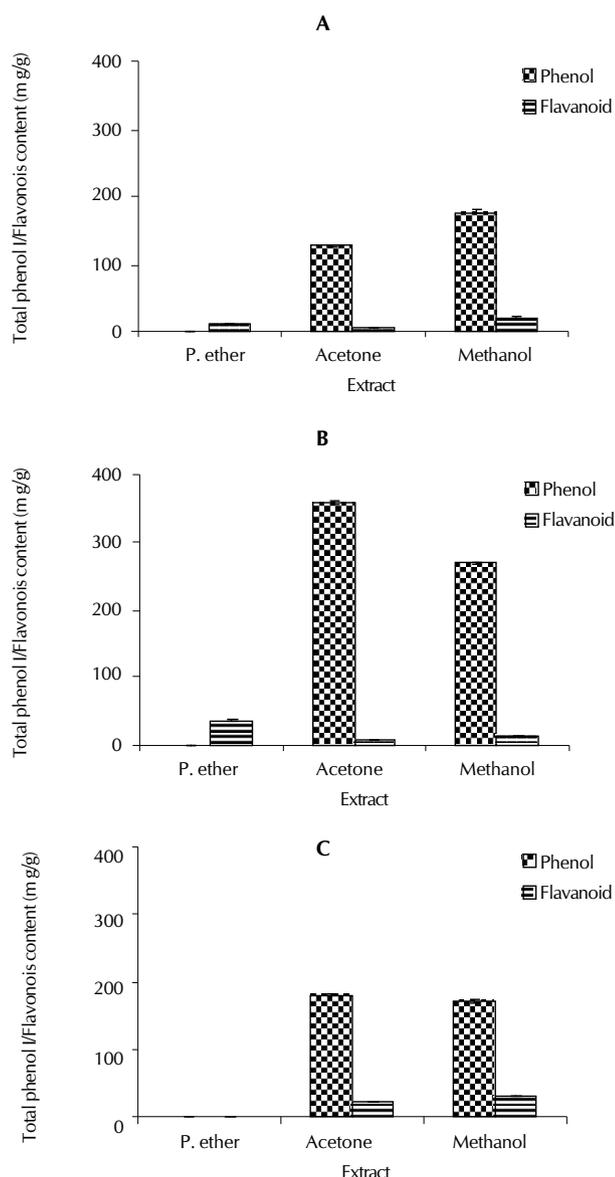


Figure 5: Total phenol and flavonoid content of different solvent extracts of *P. fraternus* A, *T. rotandifolia* B and *Z. nummularia* C.

ether extract. Flavonoid content was maximum in methanolic extract and minimum in acetone extract (Fig. 5A). In different extracts of *T. rotandifolia* total phenol content was maximum in acetone extract and minimum in petroleum ether extract. Flavonoid content was maximum in petroleum ether extract and minimum in acetone extract (Fig. 5B). In different extracts of *Z. nummularia*, total phenol content was maximum in acetone extract and minimum in petroleum ether extract. Flavonoid content was maximum in methanolic extract and minimum in petroleum ether extract (Fig. 5C).

In all the twelve plants, methanolic extract of *Peltophorum ferrugineum* and acetone extract of *Triumfetta rotandifolia* showed better total phenol and flavonoid content. Polyphenols function in trapping and scavenging free radicals due to their antioxidant properties. They also regulate nitric oxide,

decreasing leukocyte immobilization, inducing apoptosis, inhibiting cell proliferation and angiogenesis and exhibit phytoestrogenic activity (Arts and Hollman, 2005). There are claims that phenolics compounds and its derivatives are strongly correlated with antioxidant activities (Maisuthisakul *et al.*, 2007).

CONCLUSION

From the present study, it can be concluded that, extractive yield depends on the polarity of the extracting solvents. Maximum yield was obtained from the polar solvents as compared to non polar solvents. Highest extractive yield was obtained from methanolic extract of *P. ferrugineum*. Highest total phenol and flavonoid content was present in the methanolic extract of *P. ferrugineum*. Hence this plant can be a good source of polyphenolic compounds.

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