IJPSR (2021), Volume 12, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 13 January 2020; received in revised form, 15 April 2020; accepted, 20 April 2020; published 01 January 2021

IN-VITRO EVALUATION OF BIOACTIVE COMPOUNDS FROM FIVE DIFFERENT MEDICINAL PLANTS

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Keywords:

ABTS, Antioxidants, DPPH, Medicinal plants, Phytochemicals

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ABSTRACT: The forest of India is the principal repository of a large number of medicinal and aromatic plants; they are the richest source of bioactive compounds, which are largely collected as raw materials for the manufacture of drugs and perfumery products. The present study deals with five different medicinal plants *viz*., *Polyalthia longifolia*, *Anonna muricata, Elephendopus scaber, Senna alata,* and *Hyptis suaveolens,* for identifying their chemical constituents. The qualitative and quantitative analysis was performed using methanol extracts of leaves of selected medicinal plants. Total phenolic, flavonoid, tannin content, total antioxidant capacity, DPPH scavenging activity, and ABTS assay of the extracts were determined by spectrophotometric methods. Phytochemical analysis revealed the presence of alkaloids, flavonoids, terpenoids, glycosides, steroids, and saponins. *A. muricata* and *H. suaveolens* showed higher phenolic content. *A. muricata*, *H. suaveolens,* and *P. longifolia* showed significant antioxidant activity. All the extracts exhibited varying quantities of flavonoid, tannin, and alkaloid content. The presence of various bioactive compounds in the tested plants reveals that the selected medicinal plants may be a good source for production of new drugs for various ailments.

INTRODUCTION: India is richly credited with a diverse group of medicinal plants, and they are the most valuable natural resource of bioactive compounds. A number of secondary metabolites from plants have been isolated, purified, and employed in a wide range of applications, including food, pharmaceutical, cosmetic and agricultural industries [1]. Therefore, the exploration of medicinal plants and their natural bioactive molecules has become essential to exploit the possible additional values of natural sources.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(1).470-76

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(1).470-76

Polyalthia longifolia (*P. longifolia*) belongs to the family Annonaceae, and it is considered to be medicinally important. The plant is considered to be medicinally important since it contains diterpenoids and alkaloids in various parts of it. The different phytochemicals such as alkaloids, terpenes, and flavonoids have been isolated from Polyalthia species [2]. *Annona muricata* is a small upright evergreen tree of 5-6 meter height with large, glossy, dark green leaves. The leaf, stem, root, and seed extract of *A. muricata* have antibacterial activity and act as anti-parasitical agents against numerous pathogens [3].

Elephantopus scaber is a species of flowering plant in the Asteraceae family and is commonly known as Prickly-leaved elephant's foot. *E. scaber* is a common wild weed that forms undergrowth in shady places [4]. Ethyl alcohol and acetone extract of

leaves of *E. scaber* possess promising antimicrobial activity against *Escherichia coli, Salmonella typhi, Klebsiella pneumonia, Streptococcus pyogenes, Staphylococcus aureus,* and *Candida Albicans* [5]. *Senna alata* is a traditional medicinal plant belongs to Fabaceae. It is commonly known as Ringworm Bush due to its effectiveness against ringworm. Leaf extracts of the plant showed better antifungal activity against *Candida albicans, Penicillium species, A. fumigatus Microsporumcanis,* and *Trichophyton mentagrophyte* than ketoconazole (200 mg) [6].

Similarly, the selective effectiveness of *Senna alata* extract against bacterial and fungal pathogens was reported [7]. *Hyptis suaveolens,* commonly known as "Wilayati tulsi" belongs to the family Lamiaceae and is an ethnobotanically important medicinal plant. Almost all parts of this plant are being used in traditional medicine to treat various diseases. Extracts and metabolites from this plant have been found to possess pharmacological and insecticidal activities [8]. Crude leaf extract was also used as a relief to stomach ache and was reported to have antifungal properties against pathogenic dermatophytes [9]. *H. suaveolens* possesses bioactive compounds with prominent ovicidal and larvicidal properties against eggs of *Aedes aegypti* and suitable to incorporate in the integrated vector management programme [10].

MATERIALS AND METHODS:

Preparation of Plant Extract: *Polyalthia longifolia* (Annonaceae) *Anonna muricata* (Annonaceae), *Elephendopus scaber* (Asteraceae), *Senna alata* (Fabaceae), and *Hyptis suaveolens* (Lamiaceae) were collected from Western Ghats of Karnataka, India. The collected plants were identified and authenticated by Prof. K. R. Chandrashekhar, Department of Applied Botany and Mangalore University, washed with tap water, and rinsed with distilled water to remove dust particles. The leaves were shade dried and grounded into a fine powder.

Then 5 g of each plant leaf powder was dissolved in 100 ml of methanol and kept for 24 h for cold extraction. Then it was filtered, and the residue was washed with 5ml of methanol two times. The filtrate obtained was evaporated to dryness and kept at 4 ºC for further use [11].

Qualitative Screening of Phytoconstituents: Qualitative screening of secondary metabolites was carried out using methanolic leaf extracts of different plants [12].

Test for Tannins: To 2 ml of plant extract, 2 ml of distilled water, and few drops of ferric chloride ($FeCl_3$) solution were added. A green-colored precipitate indicates the presence of tannins.

Test for Saponins: 5 ml of extract was taken in a test tube and mixed with 5 ml of distilled water. The test tube was stoppered and shaken vigorously for about 5 min and allowed to stand for 30 minutes. The formation of persistent foam indicates the presence of saponins.

Test for Alkaloids: About 3 ml of extract was mixed with 3 ml of 1% HCl on a steam bath. 1 ml of the mixture was taken separately in two test tubes.

Few drops of Dragendorff's reagent were added in one tube, and occurrence of orange, red precipitate was taken as positive. To the second tube Mayer's reagent was added and appearance of buff-colored precipitate was taken as positive test for alkaloids.

Test for Flavonoids: To 1 ml of extract, few drops of 10% lead acetate solution were added. The development of yellow precipitate indicates the presence of flavonoids.

Test for Terpenoids: About 1ml of plant extract was taken in a test tube, and 2 ml of each of chloroform and concentrated sulphuric acid was added carefully to form a layer. The presence of greyish color represents the presence of terpenoids.

Test for glycosides: To 2 ml of an extract with dilute HCl and 2 ml Sodiumnitroprusside in pyridine and sodium hydroxide solution was added, the presence of cardiac glycosides can be detected by

Formation of Pink to Blood Red Colour:

Test for Steroids:

Salkowski's Test: 1 ml Chloroform extract was shaken with 2 ml of concentrated sulphuric acid. A red color lower layer indicates the presence of steroids.

Liebermann Burchard Test: 1 ml Chloroform extract was treated with few drops of acetic anhydride, and 1 ml of concentrated sulphuric acid was added along the sides of the test tube. The formation of a reddish ring at the junction of 2 layers indicates the positive result for steroids.

Test for Phenols: 1 ml of the extract was mixed with 5 ml of distilled water. To this, a few drops of neutral 5% ferric chloride solution was added. The development of a dark green color indicated the presence of phenolic compounds.

Test for Carbohydrates:

Fehling's Test: 2 ml of the extract was treated with1 mL of fehling's solution a (aqueous solution of $CuSO_4$) and 1 ml of Fehling's solution B (solution of potassium tartrate) and boiled. Appearance of red brick precipitate indicates the presence of carbohydrates.

Test for Protein: 2 ml of the extract was mixed with 2 ml of Biuret reagent. The formation of a violet colour ring indicates the presence of peptide linkages of the molecule.

Test for Phlobatanins: Formation of red precipitate was observed when aqueous extract was boiled with 1% HCl, indicates the positive result.

Test for Coumarin: About 2 ml of aqueous plant extract was dissolved in 3 ml of 10% NaOH. A yellow coloration indicates the presence of coumarins.

Quantitative Analysis:

Total Flavonoid Content: To 0.5 ml of sample 1.5 ml of methanol 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1M potassium acetate, and 2.8 ml of distilled water was added and incubated for 30 min, at room temperature.

The absorbance was measured at 415 nm. The amount of flavonoid content was expressed as (µg) equivalents of quercetin/mg of sample [13].

Total Tannin Content: 20 µl of the sample was mixed with 980 µl of distilled water, 500 µl of 1% $K_3Fe\ (CN)_6$ and 100 µl of 1% ferric chloride ($FeCl_3$) and made up to 3 ml with distilled water.

After 10 min of incubation, the reaction mixture was measured at 720 nm using a UV spectrophotometer. The tannin content was expressed as µg of tannic acid equivalents/mg of extract [14].

Determination of Alkaloid: 2.5 g of sample was taken in a 250 ml beaker, and 200 ml of 10% acetic acid in ethanol was added and covered with aluminum foil. Then, the reaction mixture was allowed to stand for 4 h and filtered.

The filtrate was concentrated to one-quarter of the original volume. Concentrated ammonium hydroxide (Conc. NH_4OH) was added dropwise to the filtrate until the precipitation was complete. The solution was allowed to settle, and the precipitate was collected by filtration and weighed [15]. The alkaloid content was determined using the following formula:

Percentage of alkaloid = Final weight of the sample / Initial weight of the extract × 100

Total Phenolic Content: The Folin-Ciocalteu (FC) method was chosen to measure the total phenolic content of the plant extracts. The test was performed as described by Shoib A. Baba and Shahid A. Malik 2014 [16] with some modifications. 0.1 ml of the sample was made up to 0.25 ml with distilled water.

To this, 0.25 ml of freshly prepared (1:1) FC reagent was added and incubated for 3 minutes, followed by 0.5 ml of (20% sodium carbonate) was added and made up to 5 ml with distilled water.

The total mixture was incubated in the dark for 30 min. The optical density was measured at 760 nm using the spectrophotometer. The results were expressed as µg of gallic acid equivalents/mg of extract. All the tests were performed in triplicates.

Total Antioxidant Capacity: The total antioxidant capacity of the extracts was evaluated by the phosphomolybdenum method as described by Pierru Betu *et al,*. 2015 [17]. 0.3 ml of extract was mixed with 3 ml of reagent mixture (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and incubated at 95 ºC for 90 min.

After cooled at room temperature, the absorbance was measured at 695 nm using UV-VIS spectrophotometer against (0.3 ml) methanol blank. The total antioxidant activity was expressed as the

number of gram equivalent of ascorbic acid. All the tests were performed in triplicates.

1, 1 Diphenyl-2-Picryl Hydrazyl (DPPH) Radical Scavenging Assay: DPPH free radical scavenging assay was measured by the following method [18]. Various concentrations of (20–100 μg/ml) leaf extracts (2 ml) were taken in a series of vials containing 3 ml of 0.1 mM methanolic solution of DPPH. The test tubes were shaken gently and incubated in the dark for 30 min. The content was measured at 517 nm. Ascorbic acid was used as standard control. All the tests were performed in triplicates. Free radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

Percentage of inhibition (%) = OD of control-OD of sample / OD of control × 100

ABTS Radical Scavenging Assay: Free radical scavenging activity of plant samples was determined by ABTS radical cation decolorization assay [19]. $ABTS^+$ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 h before use. To obtain absorbance of 0.700 at 734 nm, the $ABTS^+$ solution was diluted with methanol. After the addition of 5 µl of plant extract to 3.995 ml of diluted $ABTS^+$ solution, the absorbance was measured at 30 min after the initial mixing. An appropriate solvent blank was run in each assay. All the measurements were carried out at least three times. Percent inhibition of absorbance at 734 nm was calculated using the formula,

$$ABTS^{+} \text{ scavenging activity } \% = (Ab–Aa) / Ab) \times 100$$

Where, Ab is absorbance of ABTS radical + methanol; Aa is absorbance of ABTS radical + sample extract/standard.

RESULTS:

Qualitative Phytochemical Analysis: In quail-tative study, the selected plant extracts showed the presence of different types of phytocompounds like alkaloids, flavonoids, tannins, terpenoids, carbohydrates, protein and phenol **Table 1.** These are responsible for the major source of pharmaceuticals, food additives, fragrances, pesticides, and herbicides [20].

TABLE 1: PHYTOCHEMICAL ANALYSIS OF SELECTED MEDICINAL PLANTS

Test	*P. longifolia*	*A. muricata*	*E. scaber*	*C. alata*	*H. sauveolens*
Tannins	+	+	+	+	+
Flavonoids	+	+	+	+	+
Terpenoids	+	-	-	-	-
Saponins	+	+	+	+	+
Steroids	+	+	-	+	-
Phlobatannins	+	-	+	+	+
Carbohydrates	+	+	+	+	+
Glycosides	+	+	+	-	+
Coumarins	+	-	+	+	-
Alkaloids	+	+	+	+	+
Proteins	+	+	+	+	+
Phenol	+	+	+	+	+

(+) = Present, (-) = Absent

Quantitative Analysis: The results were expressed as the mean of three measurements ± standard deviation (SD) were performed in Microsoft Excel.

Total Phenolic, Flavonoid, Tannin and Alkaloid Content: The results of the quantitative analysis of flavonoid, tannin, alkaloid and phenolic content were represented in **Fig 1**. In methanolic extracts of plants the total phenolic content was varied. *A. muricata* extracts showed the highest phenolic content (426.95 ± 0.77 mg GAE/g) followed by *H. suaveolens* (280.85 ± 1.16 mg GAE/g), *E. scaber* (255.05 ± 0.05 mg GAE/g), *S. alata* (150.86 ± 0.98 mg GAE/g).

But the lowest phenolic content was found in *P. longifolia* (132.35 ± 0.20 mg GAE/g). A higher amount of flavonoid content was found in *S. alata* (284.93 ± 1.76 mg QE/g) followed by *E. scaber* (269.44 ± 0.30 mg QE/g) and *H. suaveolens* (241.68 ± 1.71 mg QE/g) and lower in *P. longifolia* (178.26 ± 1.03 mg QE/g) and *A. muricata* (138.25 ± 1.91mg QE/gm). Tannin content was higher in *H. suaveolens* (253.52 ± 0.37 mg TAE/g) and *A.*

muricata (209.26 ± 0.34 mg TAE/g) Lower amount of tannin was found in *E. saber* (126.14±0.76 mg TAE/g), *S. alata* (155.03 ± 0.24 mg TAE/g) and *P. longifolia* (187.07 ± 0.52 mg TAE/g). Among the five plants investigated, *H. suaveolens* (69.02 ± 0.90 mg/g) showed higher alkaloid content followed by *A. muricata* (40.63 ± 0.30 mg/g). *P. longifolia* (15.17 ± 0.14 mg/g), *S. alata* (10.19 ± 0.47 mg/g) and *E. scaber* (3.04 ± 0.05 mg/g) showed very low amount of alkaloid content.

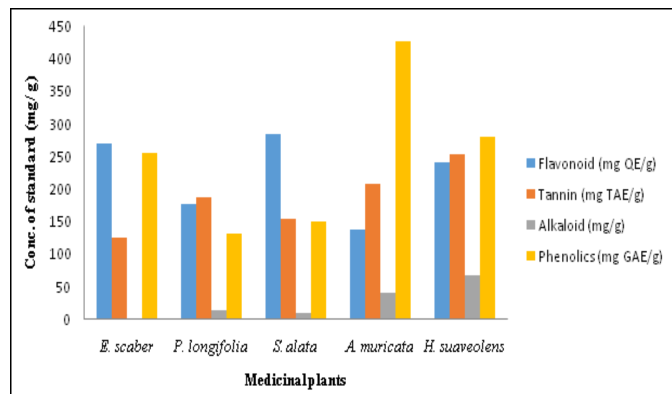

FIG. 1: QUALITATIVE ANALYSIS OF SECONDARY METABOLITES

Antioxidant Activity: Total antioxidant activity is the process of figuring out the amount of total antioxidant content present in the samples**.** The assay was depending on the reduction of Phosphate-Mo (VI) to Phosphate Mo (V) by the sample and formation of bluish-green colored phosphate/Mo (V) complex at acidic pH. Some phenolic compounds and flavonoids possess antioxidant activities because they are able to act as scavengers of singlet oxygen and free radicals in biological systems [21]. The total antioxidant activity was found to be higher in *H. suaveolens* (550.47 ± 1.09 mg/g) followed by *A. muricata* (497.19 ± 0.86 mg/g) and *P. longifolia* (453.71±1.8mg/g) and lower in *E. scaber* (318.1 ± 1.61 mg/g) and *S. alata* (353.3 ± 1.08 mg/g). The free radical-scavenging activity by DPPH aims to measure the capacity of the extracts to scavenge the stable radical DPPH formed in solution by donation of a hydrogen atom or an electron. The reduction of DPPH radical done by antioxidants is determined by the decrease of its absorbance at 517 nm. Because of the reaction between antioxidant molecules and free radicals, the change in color from purple to yellow shows the scavenging of the radical by hydrogen donation [22]. In DPPH free radical scavenging activity, the results were expressed in terms of percentage of inhibition (%). *P. longifolia* showed strong inhibition of DPPH radical (93.47%) followed by *A. muricata* (92.29%) *E. scaber* (89.85%) *S. alata* (86.16%) and *H. suaveolens* (66.08%).

ABTS assay is preferred to estimate the antiradical capacity of both hydrophilic and lipophilic antioxidants since it can be used for both organic and aqueous solvent system when compared to other antioxidant assays. This method was depending on the capacity of antioxidants to reduce the ABTS radicals [23]. ABTS percentage was found to be higher in *P. longifolia* (73.59%) and *S. alata* (73.28%) followed by *A. muricata* (61.21%). *E. scaber* (43.98%) and *H. suaveolens* (34.67%) showed the least amount of ABTS activity.

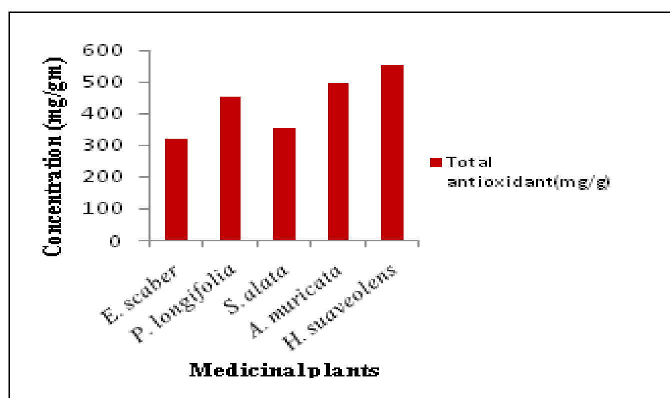

FIG. 2: TOTAL ANTIOXIDANT ACTIVITY

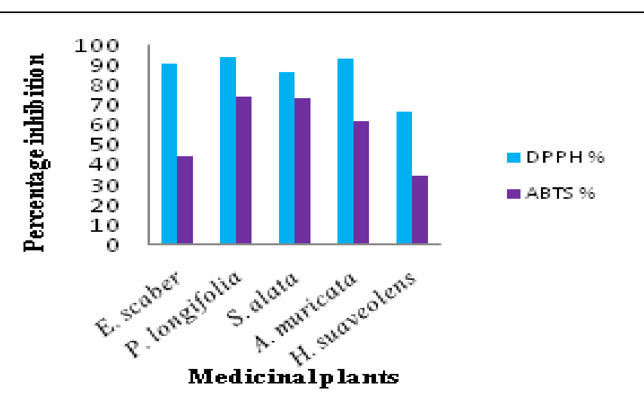

FIG. 3: ANTIOXIDANT ASSAY BY DPPH AND ABTS

DISCUSSION: Phenolic compounds, tannins, flavonoids, and alkaloids are considered to be the major secondary metabolites produced by the plants. Some of the diverse biological activities may be related to phenolic compounds. It is well known that phenolic compounds contribute to the quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing

beneficial health effects. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores [24]. All five selected medicinal plants were found to possess phenols, flavonoids, and tannins. *H. suaveolens* and *A. muricata* were rich in tannin and phenolic contents. Tannin interacts with the membrane structure of certain microorganisms and decreases the permeability of the cell membrane; thus, it helps in the inhibition of growth of microflora but not its destruction. Flavonoids act as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity [25,] which is significantly present in *S. alata* and *E. scaber*.

Terpenoids can be used as protective substances in storing agricultural products as they are known to have insecticidal properties [26]. But it was present only in *P. longifolia*. Numerous studies have confirmed that saponins possess the unique property of precipitating and coagulating red blood cells [27] steroids are responsible for cholesterol-reducing properties and regulating the immune response [28]. Saponins are present in all the plants studied, and steroids are absent only in *E. scaber*. Phlobatannins have been reported to possess astringent properties [29,] and it was found only in *A. muricata* out of five investigated plants. Plants containing carbohydrates, glycosides, and coumarins are known to exert a beneficial action on immune system by increasing body strength and hence are valuable as dietary supplements. Carbohydrates were found to be present in all the plants. Glycosides and coumarins are absent in *S. alata* and *A. muricata*. Proteins are the building blocks of life. The body needs protein to repair and maintain itself. Since it was present in all the five plants, therefore nutritional power of these plants as protein supplements cannot be ignored [30].

The total antioxidant capacity (TAC) was based on the reduction of Mo (VI) to Mo (V) at acidic pH in the presence of antioxidant components in extracts. It evaluates both water-soluble and fat-soluble antioxidants. In the present study, higher concentration of total antioxidant capacity was observed in *H. suaveolens*, *A. muricata* and *P. longifolia* extracts.

DPPH radical scavenging method has been used to determine the antioxidant activity of biological compounds. The present study reveals that the extracts of *P. longifolia, A. muricata,* and *E. scaber* were good free radical scavengers and have the ability to inhibit autoxidation of lipids. Hence it is beneficial in the treatment of various diseases where lipid peroxidation is an important mechanism for pathogenesis [31, 32].

When the ABTS reacts by losing an electron, the product obtained quickly reacts with ethanol or hydrogen donors to form a colorless ABTS. This reaction is pH-independent. A decrease in the concentration of the product obtained is linearly dependent on the antioxidant concentration. The leaf extracts of in *P. longifolia*, *S. alata,* and *A. muricata* showed potential scavenging activity for ABTS cation.

CONCLUSION: The presence of phytochemicals such as total phenolics, alkaloids, flavonoids, tannins, and antioxidants in selected plants provides some scientific evidence for the biological activities and also accounts for the pharmacological use of the plants. The present data would certainly help to ascertain the potentiality of the selected plants for medicinal use and also possess high antimicrobial properties. Therefore, further research is required for the isolation and identification of the primary and secondary metabolites, to elucidate its properties towards a potential role in the biological activity.

ACKNOWLEDGEMENT: The authors are thankful to the Department of Biosciences, Mangalore University, Mangalagangothri, Karnataka, India, for providing laboratory facilities to carry out the work.

CONFLICTS OF INTEREST: The authors declared that there is no conflict of interest.

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How to cite this article:
Purushothaman D and Chandra M: *In-vitro* evaluation of bioactive compounds from five different medicinal plants. Int J Pharm Sci & Res 2021; 12(1): 470-76. doi: 10.13040/IJPSR.0975-8232.12(1).470-76.

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