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Pharmacological and Phytochemical Screening of *Aegle marmelos* (L.) and *Cinnamomum tamala* (Buch.-ham.) Leaves for Therapeutic Efficacy

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Abstract: Medicinal plants contain bioactive components like phytochemicals, mineral elements and other pharmacological properties such as swelling and foaming index, which possess medicinal property which have therapeutic efficacy against the diseases and disorders. In present study swelling index of *A. marmelos* leaf sample (400 ± 3.6%) is significantly (p<0.001) higher than *C. tamala* (100 ± 3.5%) leaf sample and the foaming index of *A. marmelos* leaf sample (111.11 ± 2.5%) is significantly (p<0.001) higher than *C. tamala* (46.29 ± 3.1%). Among the trace mineral elements phosphorus content of both plants (28.5 ± 0.2 mg/100g and 62.10 ± 4.2 mg/100g of *A. marmelos* and *C. tamala* respectively) significantly (p<0.001) is higher and sodium content (0.3 ± 0.02mg/100g and 0.6 ± 1.4mg/100g of *A. marmelos* and *C. tamala* respectively) is significantly (p<0.05) lower among all the studied trace elements. *A. marmelos* and *C. tamala* leaf sample contain polyphenols significantly (p<0.001) highest (6.7 ± 0.61 g/100g and 16.7g/100g of *A. marmelos* and *C. tamala* leaf respectively) and flavonoids in lowest quantity (0.9 ± 0.25 g/100g and 1g/100g of *A. marmelos* and *C. tamala* leaf) among all the studied phytochemicals. Reducing power of *C. tamala* significantly (p <0.005) higher (0.72, 0.74 and 0.77%) than *A. marmelos* leaf extract (0.47, 0.55 and 0.57). Both the leaf extracts posses significantly (p < 0.005; and p <0.05 for *C. tamala* and *A. marmelos*) good reducing power as compared to the ascorbic acid.

Key words: Phytochemicals · Pharmacological · Mineral elements · Antioxidant

INTRODUCTION

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs due to presence of various types of bioactive phytochemicals, essential mineral elements and other pharmacological properties [1, 2].

The World Health Organization has listed 21,000 plants, which are used for medicinal purposes all over the world [3], among them more than 2500 species are found in India, out of which 150 species are used commercially on a large scale in pharmacological industries [4,5].

Now a days about 80% of the developed countries used traditional medicine,which has compounds derived from medicinal plants [6] and more than 30% of the modern pharmacological drugs are derived directly or indirectly derived from plants and the plants are the cheapest and safer alternative sources of drugs [7, 8].

Free radicals, reactive oxygen species (ROS) and reactive Nitrogen Species (NOS) such as superoxide (O_2•-), hydroxyl (OH•), nitric oxide (NO•), nitrogen dioxide (NO_2•) etc. are continuously produced by the body via enzymatic and non-enzymatic reactions like respiratory chain reaction, the phagocytosis, prostaglandin synthesis, phosphorylation in the human body [9, 10] but their excessive production occurs during pathogenic attack, diseases and tissue injuries, exposure to radiation etc[11].

Free radical at high concentrations damage cell structures, nucleic acids, lipids and proteins [12] and involves in the pathogenesis of many human diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, atherosclerosis, ischemic heart disease, cardiac hypertrophy, bronchopulmonary, dysplasia, intraventricular hemorrhage, glomerulonephritis, tubulointerstitial nephritis, chronic renal failure lung cancer, leukemia, breast, ovary, rectum cancers, diabetes, skin lesions, immunodepression, liver disease, pancreatitis etc [13, 14].

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Chemotherapy by synthetic drugs has been one of the most important effective medical achievements use against diseases since their introduction. However synthetic drugs are sometimes associated with adverse effects on the host, including hypersensitivity, immune suppression and allergic reactions and initiation of oxidative stress [15, 16].

The screening of plant materials and their products for pharmacological activity has shown that higher plants represent a potential source of novel therapeutic prototypes and the selection of crude plant extracts for screening program is potentially more successful in initial steps than the pure compounds [17, 18]. Natural products, either as pure compounds or as standardized plant extract, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity [19].

Aegle marmelos (L.) commonly known as Bael, belonging to the family Rutaceae. The leaves, roots, bark, seeds and fruits are edible and medicinal values. The leaves of *Aegle marmelos* are astringent, a laxative, expectorant and useful in treatment of ophthalmia, deafness, inflammations, diabetes, diarrhea, dysentery, cardiac diseases, asthmatic complications, pancreatic disorder, hepatic disorder and antimotility action on spermatozoa [20, 21]. Although this plant is native to India it is also widely found throughout the Indian peninsula and in Ceylon, Burma, Thailand and Indo-China [22, 23].

Cinnamomum tamala belonging to the family lacunaceae commonly called Tej patta comprises 270 species which occurs naturally Asia and Australia. In India it found in Sub-Himalayan tracts to West Bengal, in central and south India. It found almost in all the states of India [24, 25]. It has been used in traditional medicines as an astringent, stimulant, diuretic, carminative and in cardiac disorders, antidiarrheal, hypoglycemic activity, anorexia, dryness of mouth, bladder disorders, acaricidal, hepatoprotective, anti-inflammatory, anti-hyperlipidemic and antioxidant etc. [26].

Therefore the present study has been under taken to screen major phytochemical, mineral elements composition, reducing power and other pharmacological property of *A. marmelos* and *C. tamala* leaf.

MATERIALS AND METHODS

Collection of Plant Materials: The fresh and tender leaves were collected, dried in a shade under room temperature for six to seven days and then crushed into coarse powdery substance by using electric grinder. The coarse powdery substance was dried again and was then sieved to get fine powder using the fine plastic sieve, which was then stored in an air tight bottle in the laboratory until required [27].

Determination of Swelling Index: 1 g of powder was placed into 25 ml measuring cylinder. 25 ml of water was added and shaken thoroughly in every 10 min for 1 h. and then allowed to stand for 3 h. at room temperature. The volume occupied by the plant material was measured and compared to that of the dry powder [3, 28].

$$Swelling\ Index = \frac{Y - X}{X} \times 100$$

where, X = initial volume and Y = final volume.

Determination of Foaming Index: One gram of powder was taken in a 500 mL conical flask containing 100 mL water and boiled for 30 min, cooled and filtered into a 100 mL volumetric flask and made the volume with water. The decoction was poured into 10 test tubes in successive portion of 1mL, 2 mL, 3 mL and so on, up to 10 mL. and adjusted the volume of each test tube with water to 10mL. The test tubes were shaken length wise for 15 sec and allowed to stand for 15 minutes and the height of foam was measured and foaming index was calculated using following formula as suggested in the Quality control methods for medicinal plant materials [3, 29].

$$Foaming\ Index = \frac{1000}{a}$$

where, a = is the volume of decoction used for preparing the dilution in tube.

Extract Preparation: 50g of the sieved powder was weighed accurately and subjected to extraction in a Soxhlet apparatus at room temperature using ~350 mL distilled water. The extract obtained was filtered, concentrated after dryness in rotary flash evaporator maintained at 45°C, percentage yield of each extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies [30].

Estimation of Mineral Elements: For determination of mineral (Na, Cr, Cu, Fe, Mg, K, P, Se and Zn) contents of *A. marmelos* and *C. tamala* leaf the sample was prepared by 2g of plant material was digested with a mixture of concentrated nitric acid, sulphuric acid and perchloric acid (10:0.5:2, v/v) and the analysis was conducted using the All the glassware was cleaned by soaking overnight in a

10% nitric acid solution and then rinsing three times with deionised water [31, 32] following Atomic absorption spectroscopy with the AAS Perkin Elmer01 [33, 34].

Phytochemical Analyses: Qualitative phytochemical screening of *A. marmelos* and *C. tamala* leaf sample were conducted following Sofowara [35]. The quantitative phytochemicals analysis of detected phytochemical were done following Dandapat *et al.* [36].

Reducing Power: Spectrophotometric quantitation method was used for the determination of reducing power activity. 2.5 mL of each of the extracts was mixed with 2.5 mL of phosphate buffer (0.2M, pH 6.6) and 2.5mL of 1 % potassium ferricyanide (10 mg/mL). The mixture was incubated at 50°C for 20 min and cooled down. Then 2.5mL of 10 % trichloroacetic added to the test tube and centrifuged at 6500 rpm for 10min. An aliquot (2.5mL) of supernatant was diluted with distilled water (2.5mL) and then ferric chloride (0.5mL, 0.1 %) was added and allowed to stand for 10 min. The absorbance was recorded spectrophotometrically at 700 nm. Ascorbic acid was used as standard [37, 38].

Statistical Analysis: All results were expressed as mean ± standard error of mean (S. E. M.). Data were analyzed using Student's t-test; $p< 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Swelling and Foaming Index: Results of swelling and foaming indices are presented in Table 1. The results showed swelling index of *A. marmelos* leaves sample is significantly ($p<0.001$) higher than *C. tamala* leaf sample (Table 1). Swelling index of *T. cordifolia* stem was much higher (400%) compare to higher polymers such as pectin (55%) and xanthan (44%), which represents the drug release rate of the plant material is very high [28]. The release of drug occurs due to diffusion, dissolution and erosion mechanisms. On coming in contact with water, hydrophilic matrices undergo gel formation and progressive phase transition from glassy to rubbery state occurs. This results in salvation of individual polymer chains. As the swelling continues, the swollen matrix retains more water until the shear forces in the dissolution medium disentangle the individual polymer chains from the matrix [29, 39]. Medicinal plants are known to contain saponins that cause persistent foam when an aqueous decoction is shaken, which is indicated by the foaming index [3]. Foaming index of *A. marmelos* leaf sample was significantly ($p<0.001$) higher than *C. tamala* (Table 1). Kumar *et al.* [28] reported the foaming index of *T. cordifolia* stem was 111.12 ± 2.1 % and [29] reported 90 ± 3.54 % foaming index of *A. vasica* leaf sample. Similar results on swelling and foaming index were obtained in the present investigation. Therefore, *A. marmelos* and *C. tamala* leaf possess good pharmacological efficacy.

Table 1: Swelling and Foaming Indexes of *A. marmelos* and *C. tamala* leaf (M±SD; n=3)

Plants	Swelling Index %	Foaming Index %
Aegle marmelos	100 ± 3.5*	111.11 ± 2.5*
Cinnamomum tamala	400 ± 3.6*	46.29 ± 3.1 *

* = ($p<0.001$)

Table 2: Proximate composition of trace mineral elements from *A. marmelos* and *C. tamala* leaf (M±SD; n=3)

Mineral Elements	*A. marmelos*	*C. tamala*
Zinc	6.8 ± 0.05*	8.4 ± 0.05*
Iron	21.7 ± 0.5*	10.7 ± 1.3*
Chromium	17.4 ± 0.3*	0.4 ± 0.03*
Magnesium	0.7 ± 0.02*	60.7 ± 1.8*
Copper	1.2 ± 0.03*	0.6 ± 0.02*
Phosphorus	28.5 ± 0.2*	62.10 ± 4.2*
Selenium	1.3 ± 0.01*	0.5 ± 0.01*
Potassium	2.7 ± 0.01*	13.4 ± 2.7*
Sodium	0.3 ± 0.02**	0.6 ± 1.4**

* = ($p<0.001$); ** = ($p<0.05$)

Estimation of Mineral Elements: The results of proximate composition of mineral elements of *A. marmelos* and *C. tamala* leaf sample are presented in Table 2. The results revealed that phosphorus content of both plants (28.5 ± 0.2 mg/100g and 62.10 ± 4.2 mg/100g of *A. marmelos* and *C. tamala* respectively) significantly ($p<0.001$) is higher and sodium content (0.3 ± 0.02mg/100g and 0.6 ± 1.4mg/100g of *A. marmelos* and *C. tamala* respectively) is significantly ($p<0.05$) lower among all the studied trace elements (Table-2). Bukesh *et al.* [40] estimated the major trace elements K (1920 ± 70 ug/g), Ca (1400 ± 50 ug/g), P (799 ± 17.9 ug/g), Mg (726 ± 12.6 ug/g) and Na (340 ± 60ug/g) from leaves of *C. oxyacantha.* Aliyu *et al*. [41] reported K (32.0 mg/100g), Ca (120.0 mg/100g)), Na (136.0 mg/100g), Mg (145.0 mg/100g), Fe (0.52 mg/100g) and Zn (0.014 mg/100g) in *A. differmis.* Duthie and Brown [42] said that mineral elements such as selenium, iron, copper, zinc and manganese etc. delay or inhibit oxidative damage to a target molecule by metabolizes oxidative toxic intermediates with the help of endogenous antioxidant enzymes of body such as, glutathione peroxidase, catalase and superoxide dismutase. In present investigation estimated trace

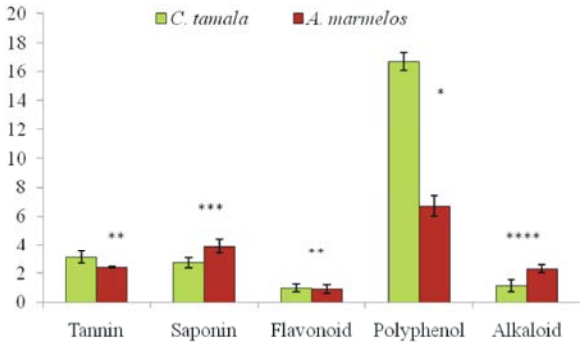

Fig. 1: Proximate phytochemical composition of leaf extract of *A. marmelos* and *C. tamala* (M ± SD; n=3); * = (p<0.001); ** = (p<0.05); *** = (p<0.01); **** = (p<0.005)

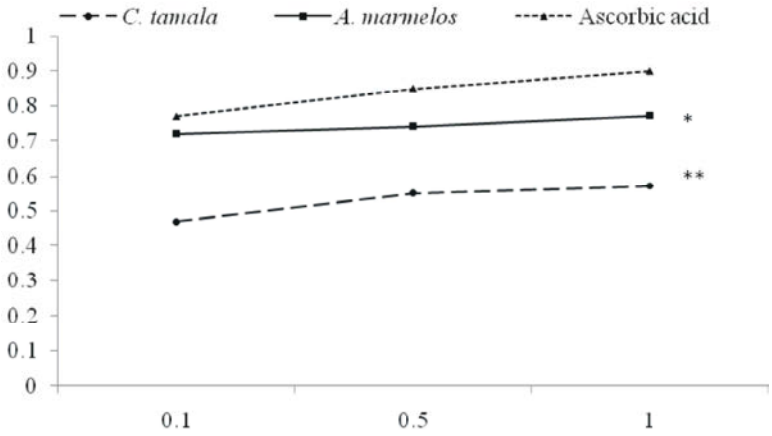

Fig. 2: Reducing power of *A. marmelos* and *C. tamala* leaf extraxt; * = (p<0.05); ** = (p<0.005)

mineral elements from *A. marmelos* and *C. tamala* leaf sample were higher than above studied plants; therefore, the leaves of both plants possess good antioxidant activity.

Phytochemical Analyses: The result of phytochemical analysis of the leaf samples of *A. marmelos* and *C. tamala* leaf sample is presented in Figure 1. The result revealed that polyphenols content is significantly (p<0.001) higher (6.7 ± 0.61 g/100g and 16.7g/100g of *A. marmelos* and *C. tamala* leaf respectively) and flavonoids occurs in lowest quantity (0.9 ± 0.25 g/100g and 1g/100g of *A. marmelos* and *C.* tamala leaf) among all the studied phytochemicals (Figure 1). Kumar *et al.* [34] reported 6.13 ± 0.13 g/100g tannin, 2.09 ± 0.17 g/100g saponin, 2.1 ± 0.21 g/100g flavonoids, 0.13 ± 0.1 g/100g poly phenols in *A. vasica*. Phenolic compounds and flavonoids, found in the edible and inedible parts of plants portray antioxidant activity and hence are of immense importance [43]. The antioxidant capacity of phenols and flavonoids is mainly due to their redox properties, which allows them to cut as reducing agents, hydrogen donors' singlet oxygen quenchers or metal chelators [44]. Alkaloids posses anti-oxidizing effects, thus reduces the nitrate generation which is useful for protein synthesis, suppresses the transfer of sucrose from stomach to small intestine [45] Dandapat *et al*. [27] reported that plant phenolics are potent inhibitors of a number of growth factor binding and signalling pathways implicated in cancer. Saponin acts as immune modulator by induce production of interlukins and interferons in human body [46]. In present study phytochemicals composition of *A. maremelos* and *C. tamala* leaf extract is higher in quantity than most of the studied above plants (Figure 1). Therefore, the leaf extract of *A. maremelos* and *C. tamala* possess good antioxidant activity.

Reducing Power: Reducing power serves as a significant reflection of the antioxidant activity. The reducing power of the test samples are compared to the standard curve of ascorbic acid (Figure 2), showing concentration-dependent. It is quite prominent that *A. marmelos* possesses significantly (p<0.005) high reducing power than *C. tamala* leaf extract. Both the leaf

extracts posses significantly ($p < 0.005$; 0.05) good reducing power as compared to the ascorbic acid. Kumar *et al.* [28] reported the concentration dependence increases in reducing power (0.1%, 0.22% and 0.23% reducing power at 0.1mg/mL, 0.5mg/mL and 1 mg/mL concentration of leaf extract) stem extract of *T. cordifolia.* Compounds with reducing power indicate that they are electron donors and can reduce the moxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [47].

CONCLUSION

Since *A. marmelos* and *C. tamala* leaves possess high swelling and foaming index, bioactive phytochemicals, mineral elements and reducing power, the leaf of both plants can be used as alternate source of synthetic medicinal supplements against diseases and disorders caused due to oxidative sterss.

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