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COMPARATIVE PHYTOCHEMICAL SCREENING AND IN VITRO ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *AEGLE MARMELOS* (L) CORR

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ABSTRACT

Context: The use of traditional medicine has increased in developed countries also, mainly due to failure of modern medicine to provide effective treatment for chronic diseases and emergence of multi-drug resistant bacteria and parasites. **Aim:** The present study was designed to detect preliminary phytochemicals and antimicrobial activity of *Aegle marmelos* (L) corr. against pathogenic strains of gram negative *Escherichia coli* (1687), and gram positive *Enterococcus fecalis* (439). **Material and methods:** The antimicrobial activity of the organic solvent extracts of *Aegle marmelos* was investigated using Cylinder -plate or Cup-plate method. **Results:** Preliminary phytochemical screening suggests that there is similarity in phytochemical profile of leaf, stem and unripe fruit. Phytochemical analysis shows that Starch, Tannin, Flavonoid and Favanol are present in higher amount in the leaves whereas Sugar and total Phenolics contents are present in higher amount in unripe fruit of *Aegle marmelos.* The present study indicated the presence of phytoconstituent like tannins, Glycoside, flavanoids, alkaloids, proteins, free amino acids, phenols and carbohydrates in *Aegle marmelos* **(leaf, stem and unripe fruit). Conclusion:** Above study illustrates that all that the leaves, stem and fruit of *Aegle marmelos* possess considerable antioxidant and antibacterial activity. Although, the leaves was found to be possess significantly higher antibacterial as well as antioxidant activity as compared with the fruit or stem. Preliminary photochemical screening suggests that there is similarity in photochemical profile of leaf and unripe fruit.

KEYWORDS: *Aegle marmelos* (L) corr., Antimicrobial, Pathogenic.

INTRODUCTION

The use of traditional medicine has increased in developed countries also, mainly due to failure of modern medicine to provide effective treatment for chronic diseases and emergence of multi-drug resistant bacteria and parasites.[1] It is believed that the standardization of plant material is not required when used by rustic communities for their primary health care. But delinquent of whether the medicinal plant to be used by local communities or by industry, an organized approach is required for a plant identified from traditional medicine, as in done in modern medicine. It is paramount to focus on all aspects of medicinal plant research from cultivation, isolation and identifying the active constituents for efficacy, evaluation, safety and formulation. So that the quality, safety and potency of herbal materials become a major interest for health authorities and pharmaceutical industries.[2] One of such medicinally important plant is *Aegle marmelos* (L) (corr) which belongs to family Rutaceae.

Aegle marmelos is scientific name of the tree which is also known as Bael in local Hindi language. English names are Stone Apple and Bengal Quince tree is one of the most useful medicinal plants of India. The medicinal properties of the plant have been described in the ancient medical memoir in Sanskrit, *Charaka Samhita*. All the parts of this tree including stem, bark, root, leaves and fruit has medicinal excellence and has been used as traditional medicine for a long time.[3] The fruit is of considerably medicinal value when it just begins to ripen. The ripe fruit is aromatic, astringent which helps in conception of skin, coolant and laxative. The unripe or half-ripe fruit is astringent, which is also involve in improving appetite and acts as ant scorbutic, i.e. it helps to fight scurvy which is caused due to vitamin C deficiency.[4]

Aegle marmelos grows in dry forests on hills and plains of central and southern India and Burma, Pakistan and Bangladesh. It is cultivated throughout India, mainly in temple gardens, because of its status as a religious tree. It is grown in some of the Egyptian gardens, and in Surinam and Trinidad.[5]

Medicinal uses of *Aegle marmelos* include anti-diabetic along with the unripe fruit or half ripe fruits are used as potent aromatic that act as cooling and laxative. Pulp or unripe fruits are astringent and are useful in diarrhea. Root and bark are given for intermittent fever, typhoid, malaria or any fever with unknown causative factor. Fruit shows laxative, anti pyretic and astringent effects. It is also useful in, hepatic fever, bronchitis, abdominal complaints, constipation, and palpitations of the heart, dysentery, piles, and seminal weakness

and vomiting. Externally it is used in conjunctivitis where juice from the leaves is placed in the eyes and paste is places over the eyelids, along with it is also used in edema and pain. Internally it is used in circulatory system as a cardiac tonic, haemostatic and alleviates swelling. The unrippened fruit is used as an appetizer and digestant while ripe fruit acts as mild laxative. Leaf juice of the plant is a liver stimulant and helps to reduce blood sugar, along with the mixture of leaf juices and black pepper is used in jaundice. The root is helpful for tranquillizing the nerves so is used in insomnia, epilepsy and hysteria. It has also usefulness in colds, cough and dyspnoea.[7]

MATERIAL AND METHODS

Plant material collection

The leaf stem and unripe fruit of plant *Aegle marmelos* (L) corr. were collected from Uttarakhand (Haldwani) region from north India. Preparation of powdered drug was done by following various steps, first of all the plant was thoroughly washed by which foreign material was identified and discarded through washing. After that plant material was dried in shed to prevent decomposition of the chemical constituents. Followed by chopping in small pieces and finally the plant material was grind till homogenous powder was formed.

Figure 1: The leaf stem and unripe fruit of plant *Aegle marmelos* (L) corr.

Preparation of plant extract

The extracts of plant were prepared by hot continuous extraction (Soxhlet) method. Extracts were prepared in different solvents like Petroleum ether, Chloroform, ethanol, Water. The large amounts of drug can be extracted with a much smaller quantity of solvent through this method. No solvent residues remain in the extract and it is an environmental friendly extraction procedure.[13]

Preliminary phytochemical screening

The phytochemical constituents present in plant exhibit great deal of medicinal importance of the drug. The present study indicated the presence of phytoconstituent like tannins, Glycoside, flavanoids, alkaloids, proteins and free amino acids, phenols and carbohydrates in *Aegle marmelos* (leaf, stem and unripe fruit). The extracts obtained from successive solvent extraction were subjected to various chemical tests to detect the chemical constituents present in them.

Test for Alkaloids

Extracts were dissolved in dilute hydrochloric acid and filtered. The filtrates were tested carefully with alkaloid reagents. Various tests were performed like Mayer's Test, Wagner's Test, Dragendorff's Test and Hager's Test for the presence of alkaloids.

Test for glycosides

Extracts were hydrolyzed with dilute hydrochloric acid and the hydrolysate was subjected to glycoside tests like Modified Borntrager's Test, Legal's Test and Balget Test.

Test for Proteins and Amino acids

Detection of Proteins and Amino acids was done by Millons Test and Biuret Test.

Test for Carbohydrates

For the detection of carbohydrates extracts were dissolved in 5ml of distilled water and filtered. Various tests were performed like Molisch's, Benedict's and Fehling's for the presence of carbohydrates.

Anti bacterial activity

Test microorganisms

The antibacterial activity was done by using bacteria gram negative strain like *Escherichia coli* (1687), and gram positive *Enterococcus fecalis* (439). All the strains were collected from Microbiology laboratory Devsthali Vidhyapeeth college of Pharmacy Rudrapur, Uttarakhand.

Preparation of the inoculums

Preparation of Media

Nutrient Agar Media it was prepared using the following composition.

Ingredients	g/l
Beef extract	10
Peptone	10
NaCl	5mg
Agar	1-2% w/v
Water	1000 ml
pH	7.3±0.1

All the ingredients were mixed and dissolved by heating. On cooling the pH was adjusted. The media was transferred in a flask and closed by cotton plug. Flasks containing media was then autoclaved at 121ºC (15 lb/sq inch) for 15 minutes.

Preparation of Plates

The plates were sterilized in autoclave for 15 minute at 15 lb/sq inch. At the same time laminar air flow exposed to UV light for 30 minutes to maintain aseptic environment. After that sterilized plates were kept on the air flow 25 ml of media was transferred in each plate aseptically. The media was allowed to solidify.

Cylinder-plate or Cup-plate method

Incorporate the various extracts in sterile cylinders or in cavities prepared in the agar. The volume of solution added to each cylinder or cavity must be uniform. Leave the dishes or plates standing for 1 to 4 hours at room temperature or at 4º, as appropriate, as a period of preincubation diffusion to minimize the effects of variation in time between the applications of the different solution. Incubate them for about 18 hours at the temperature indicated. The diameters or areas of the circular inhibition zones were measured and results were calculated.[23]

Antioxidant activity

DPPH radical scavenging assay: 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid, Rutin, methanol were used as a solvent. Standard DPPH solution was 0.135 M solution, sample stock solution was 0.1 mg/ml solution for all sample along with methanolic extract (1 mg/10 ml methanol).

Method: A solution of 0.135 M DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1 ml of extract in methanol containing 0.02-0.1 mg of the extract. The

reaction mixture was thoroughly mixed and left in the dark at room temperature for 30 minutes. The absorbance of mixture was measured spectrophotometrically at 517 nm ascorbic acid and rutin were used as references.

The ability to scavenging activity (%) = $[(ABS_{Control} - ABS_{Sample}) / (ABS_{Control})] \times 100$

Where $ABS_{Control}$ is the absorbance of DPPH radical+ methanol; ABS_{Sample} is the absorbance of DPPH radical+ sample extract.[57]

RESULT

Comparative phytochemical screening of *Aegle marmelos*

The preliminary phytochemical screening of aqueous, ethanolic, chloroform, petroleum ether, extracts of Aegle marmelos revealed the presence of cetain phytochemicals like carbohydrate, tannins, alkaloid, flavonoids etc. (Table). The presence of these compounds may account for antibacterial and antioxidant activities.

Table 1: Photochemical screening.

Photochemical screening	Leaf	Stem	Fruit
Carbohydrate	+	+	+
Tannins	+	+	+
Protein and Amino acid	+	+	+
Alkaloid	+	+	+
Flavanoids	+	+	+
Resin	+	+	+
Glycoside	+	+	+

Table 1.2: Antibacterial activity.

Microbial stain/MTCC code (gm +ve/-ve)	Zone of inhibition							
	Leaf extract		Stem extract		Fruit			Standard drug
	Aqueous	Ethanol	Pet. Ether	Ethanol	Aqueous	Ethanol	Chloroform	
Bacteria	1.2	1.3	1.0	1.1	1.1	1.2	0.9	(Ciprofloxacin)
Escherichia coli								4.6
Fungi	1.3	1.2	0.8	1.0	1.2	1.2	1.1	(Fluconazole)
Aspregillus niger								4.0

Table 2: Absorbance at various concentrations.

Conc (mg/ml)	0.02 (mg/ml)	0.04 (mg/ml)	0.06 (mg/ml)	0.08 (mg/ml)	0.1 (mg/ml)
Ascorbic acid	0.135	0.133	0.114	0.009	0.166
Rutin	0.217	0.182	0.202	0.211	0.168
***Aegle marmelos* leaf**	0.331	0.284	0.302	0.312	0.157
***Aegle marmelos* fruit**	0.258	0.153	0.189	0.213	0.187

Table 2.1: %Inhibition of DPPH by samples.

Conc. (mg/ml)	0.02 (mg/ml)	0.04 (mg/ml)	0.06 (mg/ml)	0.08 (mg/ml)	0.1 (mg/ml)
Ascorbic acid	71.2	79.4	81.1	82.2	80.1
Rutin	60.3	69.1	68.2	67.3	75.8
***Aegle marmelos* leaf**	23.1	19.4	25.2	29.3	40
***Aegle marmelos* fruit**	11.5	9.5	10.3	15.6	19.5

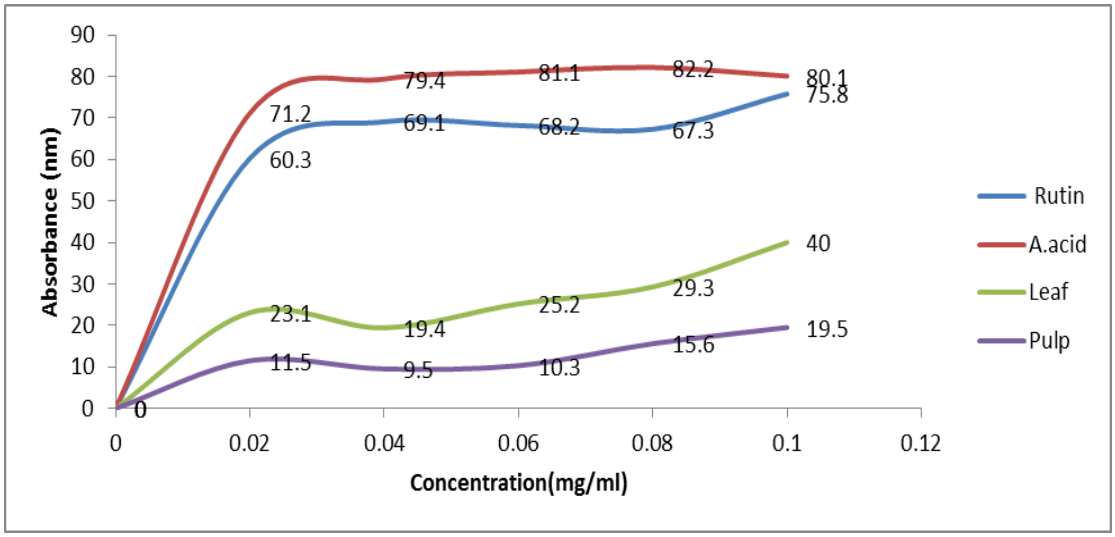

Figure 2. DPPH % free radical inhibition Vs Concentration mg/ml.

CONCLUSION

It is quite evident from review that *Aegle marmelos* contains a number of phytoconstituents which reveals its uses for various therapeutic purposes. The Plant or its individual parts can be used for the treatment of, fungal infection, microbial infection. Above study illustrates that both the leaves and fruit of *Aegle marmelos* possess considerable antioxidant and antibacterial activity. Although, the leaves was found to be possess significantly higher antibacterial as well as antioxidant activity as compared with the fruit. Preliminary photochemical screening suggests that there is similarity in photochemical profile of leaf, stem and fruit. It can be concluded from the above study that the leaves and the fruit having considerable antioxidant activity may be due to presence of higher amount of total flavonoid content and the leaves having much more antioxidant activity when compare with pulp with fruit.

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