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Qualitative Screening, Quantitative Analysis of Primary and Secondary Metabolites of Some Indogenous Herbs: *Aegle Marmelos, Annona Squamosa*

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ABSTRACT

Objective: The study includes phytochemical screening and quantification of primary and secondary constituents like carbohydrates, protein, lipids, phenol, tannin and flavonoids from Aegle *marmelos* and Annona *squamosa*. For these purpose ethanolic extract of bark was prepared by "Soxhlet extraction method". The result of these study suggests that the Aegle *marmelos* and Annona *squamosa* in which presence of primary and secondary metabolites and showed pharmacological activity like anti-microbial and anti-bacterial activity.

Keywords: Phytochemical screening**,** Primary and Secondary metabolites, Aegle *marmelos* and Annona *squamosa* leaf.

INTRODUCTION

Since long time ago, people have been observing the nature particularly medicinal plants in search of new drugs. Medicinal plants are used by 90% of the world inhabitants for their essential health needs. India is the birthplace of the transformed system of indigenous medicines such as Siddha, Ayurveda and Unani. Ancient systems of medicines are prepared from a single plant or combinations of more than one plant. This efficacy depends upon the current knowledge about various features of plant species, plant parts and pharmacological property of medicinal plants which in turn depends upon the happening of primary and secondary metabolites. Plant synthesize a wide range of chemical compounds which are classified based on their biochemical class, biosynthetic origin and active constituents into primary and secondary metabolites. Primary constituents directly involved in growth and development while secondary metabolites are not occupied directly and they have been worked as biocatalysts. Primary constituents are widely distributed in nature, occurring in one form or another in virtually all organisms. They are like chlorophyll, amino acids, nucleotides, carbohydrates etc. which have a key role in metabolic processes such as photosynthesis, respiration and nutrient assimilation. They are used as production raw materials and food excipients. The drugs selected for this work were Aegle *marmelos* and Annona *squamosa* these both important herbs are reported to have significant antibacterial, antimicrobial, antiallergic, immunomodulatory and anti-inflammatory activities which are complementary to antimicrobial activity. The growing order of natural and herbal medications, easy availability of raw materials, cost-effectiveness and the paucity of reported adverse reaction [1].

MATERIALS AND METHODS

Materials: The plants were selected on the basis of their phytochemical screening, qualitative analysis of primary and secondary constituents and their medicinal uses mentioned in the literature. The herbs (Aegle *marmelos* and Annona *squamosa*) were purchased from the forest area of the Dist. yavatmal India and authenticated by Taxonomist in the department of Botany, Shri Shivaji Science and Arts College Chikhali. All other chemicals were of analytical scale and used without further purification processes.

Preparation of extract: The powder of Aegle *marmel* leaf and Annona *squamosa* leaf were used for extraction. The powder is extracted in soxhlet apparatus with ethanol. The extraction procedure were carried out till a sufficient quantity of extract was obtained. The solvent was removed by distillation method.

SCREENING OF PRELIMINARY PHYTOCHEMICAL

Test for Alkaloids
To 5 ml of crude extract was added with 1% HCl, 3-5 drops of Mayer's reagent and Dragendorff's reagent. The alkaloids are confirmed by the organic precipitate formation.

Test for Flavonoids
The extract was added to 5 ml of dilute NH3 solution and then added to concentrated H2SO4 solution. Finally, the yellow coloration confirms the presence of flavonoids.

Test for Terpenoids
To 5 ml of test extract was mix with 4 ml of chloroform and 3 ml of con. sulfuric acid. The dark red color layer formation confirmed the terpenoids.□

Test for Steroids
In 2 ml of acetic anhydride solution was mix to 500 mg of extract with 2-4 ml of H2SO4. The steroids were confirmed by violet color change to blue.

Test for Tannins
To 5 ml of plant extract were added to 3 ml of 1% lead acetate solution. After gentle shaking, the yellow color precipitation confirms the presence of tannins.

Test for Phenols
To 5 ml of extract were added with aqueous 5% FeCl3 and formation of dark blue or black color indicates the positive of phenols.

Test for Saponins
To 5 ml of extract and 10-15 ml of distilled water was shake in a test tube upto 10 minutes. The saponins are confirmed by the formation of honeycomb structure.

Test for Proteins
In 2 ml of extract were mixed with 2 or 3 drops 1% CuSO4 solution and 1 ml of 40% NaOH solution. Finally, the violet color indicates the protein confirmation.

Test for Amino acids
In 2 ml extract were added in 2-3 ml of ninhydrin reagent and it was kept in water bath upto 20 minutes. Thereafter, turned purple color was confirms the amino acid.

Test for Carbohydrates
Two milliliters of extract and 2 drops of Molisch's reagent were added and agitate well after that 2 ml of concentrated H2SO4 was added by slowly in the test tube. The carbohydrates may confirm by the red based violet ring and formation of two layers.

No	Plant constituents/ phytochemicals and testing methods	Aegle *marmelos*	Annona *squamosa*
1	Alkaloids: Mayer's test	++	++
2	Wagner's test	++	++
3	Coumarins: Aromatic odour	+	-
4	Filter paper test	+	+
5	Saponin: Foam test	+	-
6	Heamolytic test	-	-
7	Steroids: Salkowaski reaction	-	+
8	Leberman Buchard reaction	+	+

Test microorganisms

Bacterial and fungal isolates used in the present study (bacteria*: Staphylococcus aureus*) were obtained from Hi Media Laboratories Pvt. Ltd. Navi Mumbai, culture collections of microbiology departments of P.Wadhwani college of Pharmacy, yavatmal India. The bacterial isolates were first subcultured in a nutrient broth and incubated at 37 ^{0}c. for 18 h while the fungal isolates were subcultured on a Sabouraud dextrose agar for 72 h at 25 ^{0}c.

Antimicrobial activity

The antimicrobial sensitivity patterns for the extracts were studied by disc diffusion method [2]. Sterile discs (6 mm) prepared from Whatmanís filter paper no. 1 were made to absorb (500 µg) of the test samples. Discs were left to dry under laminar flow cabinet overnight. Standard reference antimicrobial discs with cefuroxime (30 µg) for bacteria and fluconazole (10 µg) for fungal species were used as positive control and solvent discs were used as negative control. The microbial isolates were first developed in a nutrient broth for 18 h before use and standardized to 0.5 Mc Farland standards. Mueller-Hinton agar was prepared on the plates as the medium for the test organism. The microbial inoculum was spread regularly onto the surface of agar plate using the sterile cotton bud and then the extracts discs, 20% DMSO impregnated discs and standard antimicrobial discs were positioned on the inoculums agar surface. The antimicrobial activity was interpreted from the size of diameter of zone of inhibition measured to the nearest mm as observed from clear zone surrounding the disc. Each extract was assayed in triplicate and the mean of the three values was taken.

Determination of minimal inhibitory concentration (MIC)

The minimal inhibitory concentrations of dissimilar extracts were determined by two fold serial micro dilution method by means of sterile 96 well microtitre plates [3]. Hundred micro liters of the test extracts at an ultimate concentration ranging from 10 to 0.0049 mg/ml were introduced into the wells before 100 µL of standardized cell suspensions were added in each well. Microbial suspensions were used as a positive control and extract in broth was used as negative control. The MIC was taken as the lowest concentration of the extract in the well of microtitre plate that showed no turbidity after 24 h of incubation at 37OC. The turbidity of the wells was interpreted as the in evidence growth of microorganism.

Determination of minimal microbicidal concentration (MMC)

The MMC of the extracts was determined by a modification of the method of Spencer and Spencer [6]. Samples were taken from plates with no visible growth in the MIC assay and sub cultured on freshly equipped nutrient agar plates and SDA plates, later incubated at 37 ^{0}C for 48 h and 25 ^{0}C for 72 h for bacteria. MMC was taken as the concentration of the extract that did not show any visible growth on new set of agar plates.

RESULT AND DISCUSSION

The phytochemical screening demonstrated the presence of different types of compounds like alkaloids, coumarins, flavonoids and steroids which could be responsible for the antidiabetic activities. *Aegle marmelos* (leaves) extract exhibited a positive reaction to aromatic odour and filter paper test for coumarins. *Annona squamosa* (leaves) extract exhibited positive reactions to Mayer's and Wagner's test for alkaloids and Shinoda test for flavonoids.

CONCLUSION

Aegle marmelos, Annona *squamosa* leaf extracts showed antimicrobial activities against tested bacteria. Antimicrobial activities of methanol extracts and could be attributed to the presence of phenols and sterols as such activities with these compounds are reported[6]. The antimicrobial activities of methanol extract may be due to the presence of tannins, triterpenoids and flavonoids. Tannins have been known to form irreversible complexes with prolene rich protein resulting in the inhibition of cell wall synthesis[7]. Triterpenoids are known to weaken the membranous tissue, which results in dissolving cell wall of microorganism[9]. Flavonoids, another constituent of methanol extract, have exhibited a large number of biological activities like anti-inflammatory, antioxidant and antimicrobial properties [10].

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