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Phytochemical Screening of Different Solvent Extracts of Soap Berry (*Phytolacca dodecandra* L'herit.)- A Native Ethiopian Shrub



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ABSTRACT

Medicinal plants are major remedy for a variety of diseases and have been used since time immemorial. *Phytolacca dodecandra* L'Herit (Endod: Amharic) is an African soapberry that grows as shrub or climber native to Ethiopia and Eritrea. The aim of the present study was to screen the various phytochemicals from the benzene, CCl₄, hexane, and aqueous extracts of leaves, fruits and stem of *P. dodecandra*. All solvent extracts were investigated to qualitative preliminary phytochemical screening using prescribed methods. The results showed that the plant has various secondary metabolites like alkaloids, protein and amino acids, saponins, flavonoids, terpenoids and total phenols and tannins. The phytochemicals generated data from the four different extracts of *P. dodecandra* may be used as tools for quality control of drugs in the future, for the healing of a diversity of disease conditions.



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INTRODUCTION

The beneficial efficacy of most aboriginal plants for an assortment of diseases has been described by conventional herbal medicinal practitioners since time immemorial. Natural products are the base of synthetic and traditional herbal medicine, which are extremely protected as well as environment-friendly. According to WHO, over 75 percent of the inhabitants of developing and developed countries rely on conventional medicine for their primary healthcare needs.¹ They are bioactive chemicals of plant derivation, which are seen as secondary metabolites. Naturally, these bioactive chemicals are manufactured in all parts of the plant body, i.e., bark, leaves, stems, root, flower, fruits and seeds.² The amount and quality of bioactive constituents exist in plant parts may vary from one part to another. Indeed, the biological activity of plants is highly depending on the distribution of bioactive constituents (active principles) which are more frequent in several fraction of the plants.³ The thriving determination of active constituents screened from plant material is chiefly dependent on the range of solvent utilized in the extraction methods.² For this reason, it highlights that various solvent attempt is compulsory to screen the plant parts for bioactive compounds.

Phytolacca dodecandra L'Herit (Endod: Amharic) is an African soapberry in the order of Caryophyllales and Phytolaccaceae family. It is a perennial rock climbing herb with hanging branches growing rapidly in the highlands of Ethiopia and typically produce fruits twice in a year. Various fractions of the plant are therapeutically used to treat variety of diseases in humans and animals.⁴ Pulverized berries are generally utilized as detergents in different regions of Ethiopia.⁵ The leaves of plant play a potential mosquito larvicidal, abortion and treatment of malaria.^{6,7} The young berries have molluscicides potential.⁸ Moreover, a variety of therapeutic uses are predictable internationally includes the treatment of diuretics, abdominal cramps, edema, anthelmintics, and laxatives ringworms, scabies, dandruff, itching, rheumatism, intestinal roundworms, emesis, otitis and pneumonia.⁹⁻¹² In the present study, various solvent extracts of leaves, stem and fruits of *P. dodecandra* were qualitatively screened for phytochemicals using standard tests.

MATERIALS AND METHODS

Chemicals

Ferric chloride, HCl, Dragendorff's reagent, hexane, benzene, carbon tetrachloride, chloroform, H₂SO₄, Folin-Ciocalteu reagent were purchased from Chemico Glass & Scientific Company, Erode, Tamilnadu, India. All the chemicals used throughout this experiment were of analytical grade.

Collection and authentication of plant material

Soapberry was collected from Jimma University Garden, Jimma, South West Ethiopia in the month of October-2014. The plant has been taxonomically identified and authenticated by the Jimma University Botanist Dr. Florence and kept in Jimma University Botanical Science and Herbarium for future references.

Benzene extract of leaves, fruit and stem of Soapberry

The shadow dehydrated roughly powdered of leaves, fruits and stem of Soapberry was engrossed and haul out with benzene for 72hrs. After finishing point, the defatted solutions were sieved by filter paper Whatmann No.1 to eliminate any contamination. The extract was intensified by vacuum desiccator to reduce the degree; the concentrated samples were relocated to another beaker and the residual solvent was further vaporized. Finally, the dark greenish yellow coloured extract was formed and again it was kept in a vacuum desiccator to get rid of unnecessary wetness. Dehydrated extract was stored in sealed a container and which was used for qualitative phytochemical screening.

Aqueous, CCl₄, and Ethanol extracts of leaves, fruit and stem of Soapberry

The residues left after benzene extraction was dehydrated and then engrossed separately with aqueous, CCl₄ and ethanol respectively up to 3 days. After finishing point of extraction, the organic solvents were eliminated by vacuum desiccator. Dark greenish yellow colour extracts were formed and then kept in a sealed container for further studies.

Preliminary phytochemical studies^{13,14}

The extracts obtained (benzene, ethanol, carbon tetrachloride, and aqueous) were employed for the subsequent phytochemical screening.

Test for Alkaloids

a) Dragendorff's test: Take 1ml of the solvent extract, add equal volume of distilled water followed by 1ml of 2molar solution of HCl added until acidification reaction take place. To Add this 1ml of Dragendorff's reagent. Orange or red colour is formed, indicated that the occurrence of alkaloids.

b) Hager's Test: Take 1ml of the solvent extract in a cleaned test tube, add 1ml of Hager's reagent. Yellow precipitate is formed, indicated that the occurrence of alkaloids.

c) Wagners Test: Take 1ml of solvent extract acidified with 1ml of 1.5% v/v of HCl and add 1ml of Wagner's reagent. Formation of yellow or brown precipitate, which indicated that the occurrence of alkaloids.

d) Mayers Test: Take 1ml of Mayers reagent, add 1ml of solvent extract. White or pale yellow precipitate is formed, indicated that the occurrence of alkaloids.

Test for Carbohydrates

a) Anthrone Test: Take 1ml of solvent extract and 10ml of distilled water in a test tube, shaken vigorously and filtered. To this filtrate, add 1ml of anthrone reagent and mixed. Green or blue color is formed, indicated that the occurrence of carbohydrates.

b) Benedicts Test: Take 1ml of solvent extract and 10ml of water in a test tube, shaken vigorously and filtered. To this filtrate, add 3ml of Benedict's reagent and kept in a boiling water bath for 5min. Development of red colour indicated that the occurrence of reducing sugar.

c) Fehlings Test: Take 1ml of solvent extract and 10ml of water in a test tube, shaken vigorously and filtered. To this filtrate, add 1ml of Fehlings solution A and 1ml of Fehlings solution B and

kept in a boiling water bath for 5min. Development of red colour indicated that the occurrence of reducing sugar.

d) Molischs Test: Take 1ml of solvent extract and 10ml of water in a test tube, shaken vigorously and filtered. To this filtrate, 1ml of Molisch reagent were added followed by few drops of Conc. H_2SO_4 added in the side of the test tube. Formation of two junctions, which indicates the occurrence of carbohydrates.

Test for flavonoids

a) Shinods test: Take 1ml of solvent extract diluted with 3ml of ethanol followed by 2ml dilute HCl and pinch of Mg in a test tube, shaken gently. Appearance of pink or brown precipitate indicated that the occurrence of flavonoids.

b) With Con. H_2SO_4 test: When treated with Con. H_2SO_4 , appearance of the following colour like yellow colour (anthocyanins), yellow colour change to orange (flavones); orange colour change to crimson (flavanones) respectively.

Test for Glycosides

Molisch Test: Take 1ml of solvent extract and 10ml of water in a test tube, shaken vigorously and filtered. To this filtrate, 1ml of Molisch reagent were added followed by few drops of Conc. H_2SO_4 added on the side of the test tube. Formation of two junctions, which indicates the occurrence of glycosides.

Test for proteins and free amino acids

1. Millions test- Take 1ml of solvent extract with 1ml of Millions reagent, shake gently. Appearance of cherry red color indicated that the occurrence of free amino acid.

2. Ninhydrin test- Take 1ml of solvent extract with 1ml of Ninhydrin reagent. Shake gently formation of violet color indicated that the occurrence of free amino acids.

3. Biuret test: Take 1ml of solvent extract with 1ml of 10% NaOH and 1ml of 1% copper sulphate in a test tube, shake gently. Development of purple color indicated that the occurrence of proteins.

Test for gums and mucilage

With 95% alcohol: Take 1ml of solvent extract with 25 ml of 95% alcohol in a test tube, shake gently and filtered. The residue was air dried and studied for its bulging property. It indicated that the occurrence of gums and mucilages.

Test for anthraquinones

Take 2ml of the solvent extracts acid hydrolysed with Conc. H₂SO₄ followed by extracted with benzene. Add 2ml of dilute ammonia. Appearance of rose pink color indicated that the occurrence of anthraquinones.

Test for Saponins

Foam test: Take 5ml of solvent extracts in a test tubes add a drop of sodium bicarbonate, shaken vigorously and kept it stands for 3min. Development of cloudy white precipitate indicated that the occurrence of saponins.

Test for Sterols

a) Liebermann-Buchards test: Take 1ml of solvent extract in a test tube and add acetic anhydride and kept in a boiling water bath for 5min, then cooled followed by 1ml of Con. H₂SO₄ added along the sides of the test tube. Appearance of green color indicated that the occurrence of steroids.

b) Salkowski reaction: Add 1ml of solvent extract diluted with chloroform and followed by 1ml of Con. H₂SO₄ added along the sides of the test tube. Appearance of two junction/layer indicated that the occurrence of steroids.

Test for fixed oils

Spot test: Take 0.5ml of solvent extract and pressed in between the two filter papers. Formation of oil stains on the paper indicated the existence of fixed oil.

Add 1ml of 0.5N alcoholic KOH and 1ml of solvent extract along with a single drop of phenolphthalein in a test tube. The residues were kept in a boiling water bath for 20min. Appearance of soap or incomplete neutralization of alkali indicated that the occurrence of fixed oils.

Test for triterpenoids

Add 2ml of solvent extract and 1 ml of CHCl_3 followed by 1 ml of acetic anhydride in a test tube and shake gently. Add 1ml of Con. H_2SO_4 added along the sides of the test tube. Appearance of two junction/layer indicated that the occurrence of triterpenoids.

Test for phenolic compounds and tannins

About 5ml of solvent extracts and equal volume of water added and perform the following reagent for confirmation of phenolic compounds and tannins.

Ferric chloride reagents- It gives a violet color.

Gelatin containing sodium chloride- It gives a white precipitate.

Lead acetate solution- It gives a white precipitate.

RESULTS AND DISCUSSION

In the current study, phytochemical analysis was done in benzene, aqueous, ethanol and CCl_4 extracts of soapberry leaves, fruits and stem showed the presence of phytochemical constituents, namely alkaloids, proteins and amino acids, flavonoids, saponins, steroids, total phenols and tannins, triterpenoid, and absence of glycosides, anthraquinones, and steroids, shown in Table I.

The initial phytochemical screening tests may be helpful in the screening of bioactive compounds and eventually may help to detection and development of new drugs. Further, these

tests make easy their qualitative separation and quantitative estimation of pharmacologically active chemical compounds.¹⁵ The phytochemical screening in the present study has publicized the presence of alkaloids, flavonoids, saponins, total phenols and tannins, and triterpenoids in the leaves, fruits and stem extracts. Further, the presence of different phytochemicals in the four different organic solvent extracts may be responsible for the therapeutic properties of soapberry.

Tannins and flavonoids are phenolic compounds that are acting as principal antioxidants or free radical scavengers. Since these phenolic compounds were originated to be present in the extracts, it might be accountable for the potent antioxidant capacity of soapberry. These phytochemicals of medicinal plants have primarily reported for their medicinal value, which can be valuable for therapeutic index. For instance, saponins proved as hypotensive and cardio depressant properties,¹⁶ which are helpful for the treatment of congestive heart failure and cardiac myopathy.² The occurrence of saponins in ethanol and aqueous extracts of leaves, stem and fruits of soapberry might play a role in the cardioprotective potential. Alkaloids and tannins have the potential of hypoglycemic and anti-inflammatory activities.¹⁷ Moreover, the terpenoids have also been revealed to decrease blood sugar level in animal studies.¹⁸ In addition, the steroids and triterpenoids demonstrated the analgesic properties and central nervous system activities.¹⁹⁻²¹ Hence the preliminary phytochemical investigation are actually obliging in finding chemical ingredients in the plant that may help to their quantitative evaluation and also in locating the source of pharmacologically active principle.

CONCLUSION

The results of the phytochemical analysis showed the leaves, stem and fruits extracts of soapberry indicate their potential as a source of bioactive principles that may supply drugs for modern medicines. Further studies are therefore required to validate their antimicrobial, antihyperglycemic, anti-inflammatory, and anthelmintic activities. In addition, isolation purification and characterization of the active principles are necessary to ensure that the plant has novel interesting studies.

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