PHYTOCHEMICAL SCREENING OF VARIOUS ETHANOLIC SEED EXTRACTS


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ABSTRACT

The screening and study of four different seeds belonging to different families for phytochemical constituents was performed using generally accepted laboratory technique for qualitative determinations. The constituents screened were tannins, saponins, terpenoids, flavonoids, glycosides, carotenoids, steroids, protein, carbohydrate and alkaloids. The distribution of these constituents in the seeds were assessed and compared. The plant seeds studied were Phoenix dactylifera, Manilkara zapota, Artocarpus heterophyllus and Carica papaya. Results revealed the presence of saponins, tannins, flavonoids, alkaloids, carbohydrates, phenolic compounds and carotenoids in the ethanolic extract of Carica papaya seeds. In case of other seeds, ethanolic extract of Phoenix dactylifera contains saponin, terpenoids, phenolic compounds and glycosides. Ethanolic extracts of Manilkara zapota contains tannins, terpenoids and phenolic compounds whereas Artocarpus heterophyllus extract showed the positive results for saponins, tannins, terpenoids and flavonoids.

Key Words: Plant seeds, ethanolic extract, phytochemical constituents.

INTRODUCTION

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. This knowledge is accessible from thousands of medical texts and manuscripts. This traditional knowledge forms the codified systems of medicine and exists in the forms of Ayurveda, Unani, Siddha and Swa-riga (Tibetan) systems of medicine. The flora and fauna are used for medicinal purposes and they have important cultural roles and as well as vital roles in forest ecology, such as pollination, seed predation and dispersal, seed germination, herbivory and predation on potential pest species. Ethnomedicinal study deals
with the study of traditional medicines. Since ancient times mankind has been using herbal plants, organic materials as well as materials from the sea, rivers etc. for its betterment. These substances have been used as food, medicine etc. Amongst them, the substances having medicinal value have been extensively used for treating various disease conditions. Herbs being easily available to human beings have been explored to the maximum for their medicinal properties. Various parts of the plants like roots, leaves, bark, exudates etc. are used as per medicinal properties.

Herbal medicine is cost-effective and less expensive than the medicines bought from an allopathic pharmacy. Herbal medicine and remedies are more effective than allopathic medicine for certain ailments. The chemical medicine prescribed by a pharmacist could have certain negative side effects. However, many of the herbal medicines and remedies do not have negative side effects. If any, they are softer than allopathic medicine.

Several herbal drugs have been reported to posse’s anti-ulcerogenic activity by virtue of their predominant effect on mucosal defensive factor. The plants and herbs are used to treat different gastrointestinal illnesses, including peptic ulcer. Some polyphenolic compounds have been reported to have anti ulcerative activity. Extracted polyphenols or particular polyphenols belonging to the flavonoids family of compounds catechin, quercetion were reported to have certain efficacy in animal models. Flavonoids such as quercetin, flavones have been shown to inhibit growth of Helicobacter pylori. The fruit seeds contains rich amount of polyphenols and quercetion compounds.

**Phoenix dactylifera**

Phoenix dactylifera is a palm in the genus Phoenix, cultivated for its edible sweet fruit. Dates contain 20–70 calories each, depending on size and variety. Date seeds are soaked and ground up for animal feed. Their oil is suitable for use in soap and cosmetics. Date palm seeds contain 0.56–5.4% lauric acid. They can also be processed chemically as a source of oxalic acid. The seeds are also burned to make charcoal for silversmiths, and can be strung in necklaces. Date seeds are also ground and used in the manner of coffee beans, or as an additive to coffee. Experimental studies have shown that feeding mice with the aqueous extract of date pits exhibit anti-genotoxic and reduce DNA damage induced by N-Nitroso-N-methylurea (Diab et al., 2012).
Manilkara zapota
Manilkara zapota, commonly known as the sapodilla (Morton, 1987). The fruit is a large ellipsoid berry, normally 4–8 but up to 15 cm in diameter, containing two to five seeds. Inside, its flesh ranges from a pale yellow to an earthy brown color with a grainy texture akin to that of a well-ripened pear. The seeds are black and resemble beans, with a hook at one end that can catch in the throat if swallowed. The fruit has an exceptionally sweet, malty flavor. The unripe fruit is hard to the touch and contains high amounts of saponin, which has astringent properties similar to tannin, drying out the mouth. Compounds extracted from the leaves showed anti-diabetic, antioxidant and hypocholesterolemic (cholesterol-lowering) effects in rats (Fayek et al., 2012). Acetone extracts of the seeds exhibited considerable antibacterial effects against strains of Pseudomonas oleovorans and Vibrio cholerae.

Artocarpus heterophyllus
The jackfruit (alternately jack tree, jackfruit, or sometimes simply jack or jak; Artocarpus heterophyllus) is a species of tree in the Artocarpus genus of the mulberry family (Moraceae). It is native to parts of South and Southeast Asia, and is believed to have originated in the south western rain forests of India, in present-day Kerala, coastal Karnataka and Maharashtra. The jackfruit tree is well suited to tropical lowlands, and its fruit is the largest tree-borne fruit, reaching as much as 80 pounds (36 kg) in weight, 36 inches (90 cm) in length, and 20 inches (50 cm) in diameter (Boning, 2006). The jackfruit tree is widely cultivated and popular food item in tropical regions of India, Bangladesh, Nepal, Sri Lanka, Cambodia, Vietnam, Thailand, Malaysia, Indonesia, and the Philippines where it is known as Nangka. Jackfruit is also found across Africa (e.g., in Cameroon, Uganda, Tanzania, Madagascar, and Mauritius), as well as throughout Brazil and in Caribbean nations.

In general, the seeds are gathered from the ripe fruit, sun-dried, and then stored for use in rainy season in many parts of South Indian states. They are extracted from fully matured fruits and washed in water to remove the slimy part. Seeds should be stored immediately in closed polythene bags for one or two days to prevent them from drying out. Germination is improved by soaking seeds in clean water for 24 hours. During transplanting, sow seeds in line, 30 cm apart, in a nursery bed filled with 70% soil mixed with 30% organic matter. The seedbed should be shaded partially from direct sunlight in order to protect emerging seedlings.
Carica papaya
The papaya is a large, tree-like plant, with a single stem growing from 5 to 10 m (16 to 33 ft) tall, with spirally arranged leaves confined to the top of the trunk. The lower trunk is conspicuously scarred where leaves and fruit were borne. The leaves are large, 50–70 cm (20–28 in) in diameter. Papaya seeds have recently caught attention as a potential health food, and while much of the evidence supporting the notion is unverified, the seeds are nontoxic and might be worth trying just in case. Papaya seeds by treating them as a supplement and eating them whole, or you could grind the seeds up and use them when you would usually use pepper since the two tastes are fairly similar (Aravind, 2013).

However, there are no reports on phytochemical analysis of seed extracts of Phoenix dactylifera, Manilkara zapota, Artocarpus heterophyllus and Carica papaya. Hence, the authors have made an attempt on phytochemical analysis of seed extracts.

METHODS

Collection of seeds
The seeds were collected in and around Kanayakumari. The seeds were shade dried and made into powder by means of mechanical blenders.

Preparation of ethanol extract
The seeds were dried in hot air oven at 40-50°C for a week. The dried plant material was powdered using mixer grinder, and subjected to shoxlet extraction with 99% ethanol for 24 hrs. The mixture was evaporated and stored in refrigerator. The condensed extracts were used for preliminary screening of phytochemical constituents.

Preliminary phytochemical screening of various extracts
Preliminary phytochemical screenings of various extracts and drug powder were carried out as per the standard textual procedure (Harborne, 1973).

Test for Saponins
The seed extracts was shaken well with water and observed the formation of foam which was stable for 15 minutes indicates positive result.

Test for Tannins
The seed extracts was mixed with basic lead acetate solution would give white precipitate indicating the presence of tannins.
Test for Terpenoids (Salkowshi test)
2 ml of the ethanolic extract of seeds were dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. Development of a greyish colour indicates the presence of terpenoids.

Test for Flavonoids (Ferric chloride test)
The test solution was treated with few drops of ferric chloride solution would result in the formation of blackish red colour indicating the presence of flavanoids.

Test for Alkaloid (Hager’s test)
The solution was treated with few drops of Hager’s reagent (saturated picric acid solution). Formation of yellow colour precipitate shows a positive result for the presence of alkaloids.

Test for Proteins (Biuret Test)
Test solution with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink colour.

Test for carbohydrate (Benedict’s test)
Test solution was mixed with few drops of Benedict’s reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

Determination of total phenolic compounds
The total phenolic compounds of the samples were determined by the Folin-ciocalteau method (Amin et al., 2006). 5 grams per 50 ml of sample was filtered with what man paper no.1. The sample of 0.5ml was added to 2.5ml of 0.2 N folin-ciocalteau reagents and placed for 5 minutes. 2ml of 75 g/l of Na2CO3 were then added and the total volume made up to 25ml using distilled water. The above solution was then kept for incubation at room temperature for 2 hours. Absorbance was measured at 765 nm (Singleton et al., 1965).

Determination of total quercetin (Flavanoids) compound
Total quercetin content of the sample was determined by change method. One ml of the sample was added to one ml of 95% ethanol. 0.2ml of 10% aluminium chloride, 0.2ml of one molar potassium acetate were added and total volume made up to 5ml distilled water and 1 ml of 10% aluminium chloride was added and the above solution was then kept for incubation
at room temperature for 10 mins. Absorbance was measured at 450 nm (Singleton et al., 1965)

**Tests for glycosides: Liebermann’s test**

2 ml of the organic extract was dissolved in 2 ml of chloroform and then 2 ml of acetic acid was added in it. The solution was cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence of a steroidal nucleus (that is, a glycone portion of glycoside).

**Test for carotenoids**

1 g of each sample was extracted with 10 ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85 % sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

**RESULTS AND DISCUSSION**

Table: 1 phytochemical screening of seed extracts (n=3)

<table>
<thead>
<tr>
<th>Seeds</th>
<th>P. dactylifera</th>
<th>C. papaya</th>
<th>M. zapota</th>
<th>A. heterophyllus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>Terpenoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Flavonoids</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>Alkaloids</td>
<td>-</td>
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<tr>
<td>Proteins</td>
<td>-</td>
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<tr>
<td>Carbohydrate</td>
<td>-</td>
<td>+</td>
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<td>-</td>
</tr>
<tr>
<td>Total phenolic compounds</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>carotenoids</td>
<td>-</td>
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</table>

The study of these four different plant seed species namely Phoenix dactylifera, Manilkara zapota, Artocarpus heterophyllus and carica papaya for screening of phytochemical constituent was performed using generally accepted laboratory technique for qualitative determinations. Ethanolic extract of carica papaya seeds contains saponins, tannins, flavonoids, alkaloids, carbohydrates, phenolic compounds and carotenoids. The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported by Kubmarawa et al., 2007 and Mensah et al., 2008 reports alkaloids in 12 leafy vegetables studied. Ayitey-Smith and Addae-Mensah11 had earlier recorded that bitter leaf contains an alkaloid which is capable of reducing headaches.
associated with hypertension. In case of other seeds, ethanolic extract of Phoenix dactylifera contains saponin, terpenoids, phenolic compounds and glycosides. Ethanolic extracts of Manilkara zapota contains tannins, terpenoids and phenolic compounds whereas Artocarpus heterophyllus extract showed the positive results for saponins, tannins, terpenoids and flavonoids. Flavonoids are known to be synthesized by plants in response to microbial attack. Hence, it should not be surprising that they have been found to be effective antimicrobial substances against a wide array of microorganisms, when tested in-vitro. Their activity is probably due to their ability to react with extracellular and soluble proteins and to complex with bacterial cell walls leading to the death of the bacteria (Cowan, 2002). Tannins are also reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects. Phytotherapeutically, tannin containing plants are used to treat nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins.

CONCLUSION
Phytochemical screening of ethanolic extracts of seeds such as Phoenix dactylifera, Manilkara zapota, Artocarpus heterophyllus and Carica papaya was done. Results revealed the presence of saponins, tannins, flavonoids, alkaloids, carbohydrates, phenolic compounds and carotenoids in the ethanolic extract of Carica papaya seeds. In case of other seeds, ethanolic extract of Phoenix dactylifera contains saponin, terpenoids, phenolic compounds and glycosides. Ethanolic extracts of Manilkara zapota contains tannins, terpenoids and phenolic compounds whereas Artocarpus heterophyllus extract showed the positive results for saponins, tannins, terpenoids and flavonoids.

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REFERENCES


