Phytochemical screening and isolation of Eugenol from *Syzygium aromaticum* by gas chromatography

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ABSTRACT

Tannins, phlobatannins, saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids distribution and the isolation of eugenol from *Syzygium aromaticum*, belongs to family Myrtaceae was examined. Qualitative analysis carried out on the plant shows that tannins, saponins, flavonoids, terpenoids and alkaloids are present in the plant. The gas chromatographic analysis of the compound was reported to have retention time of 7.93 minutes, whereas the mass spectroscopic analysis showed 164 as m/z value, which was in accordance with the molecular weight of eugenol. The significance of the phytochemical constituents with the respect to the role of the plant in traditional medicine treatment is discussed.

Keywords: Eugenol; phytochemicals; *Syzygium aromaticum*

INTRODUCTION

Plant extracts or bioactive herbal compounds have been reported scientifically for their biological activities. Phytochemicals may protect human from a host of diseases. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are organic substances and could be obtained in both primary and secondary metabolic process; they also provide a source of medicine since the earliest time. The plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world’s pharmaceuticals. The most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. Plants in all facet of life have served a valuable starting material for drug development (Edeoga, 2005). Antibiotics or antimicrobial substances like saponins, glycosides, flavonoids and alkaloids etc., are found to be distributed in plants, yet these compounds were not well established due to the lack of knowledge and techniques (Hafiza,). The phyto-constituents which are phenols, anthraquinones, alkaloids, glycosides, flavonoids and saponins are antibiotic principles of plants. From these phytoconstituents, saponins have been reported to exhibit hemolytic and foaming activity (Ferroz, 1993), antifungal (Nagata, 1980), anti-inflammatory (Zehavi, 1986), fungistatic (Sati, 1987), molluscidal (Ayitey-Smith, 1977).

The medicinal properties of some plants have been documented by some researchers (Gill, 1992; Banso, 2007; Nassar, 2007). This study looks into the fundamental scientific bases for the use of some medicinal plant seeds by determining the crude phytochemical constituents present in these plants. Plants have limitless ability to synthesize aromatic substances, mostly phenols or their oxygen-substituted derivatives (Geissman, 1963). Most of the natural products are secondary metabolites and about 12,000 of such products have been isolated so far. These products serve as plant defense mechanisms against predation by micro-organisms, insects and herbivores (Fransworth and Morris, 1976).

Clove belongs to the family Myrtaceae have historically been used in Indian cuisine (both north Indian and south Indian), where it is often paired together with cumin and cinnamon. In north Indian cuisine, it is used in almost all dishes, along with other spices. It is also a key ingredient in tea along with green cardamom.

Clove buds act as antioxidants because of presence of the 16 identified volatile compounds (Singh, 2009), clove buds have been found to possess the anti-stress activity in its hydro-alcoholic extract (Gildemeister, 1989).

Clove buds have been found possess antidepressant activity, because it inhibits the monoamine oxidase A (MAO-A), and monoamine oxidase B (MAO-B), so have been found to act against Alzheimer’s disease (Tao, & Irie, 2005). Some derivatives of eugenol like (4-allyl-2-methoxyphenol) could act as anticancer substituents because eugenol and its analogues bear a chemotherapeutic role of nitro derivatives. Clove has historically been used as active pharmaceutical agent with a carminative and analgesic activity.

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Email: mohdaminmir@gmail.com
Received on: 09-08-2012
Revised on: 20-08-2012
Accepted on: 02-04-2013
MATERIALS AND METHODS

Chemicals and Equipments

All the chemicals used in the investigation were of analytical grade (AR), and were purchased from Sigma, Merck etc. Deionized water was used for the complete study. All the glass ware and equipments used were sterilized prior to use. Sterilization process was performed by autoclaving at 121°C for 15 minutes.

Extraction for phytochemical study

75g of plant powdered was weighted accurately and extracted for 14 hours, with methanol in a Soxhlet apparatus. The extract after extraction was evaporated to remove the volatile solvent and get the plant extract in solid form after being kept in an oven for complete dryness.

Chemical tests for the phytochemicals

Chemical tests were carried on the all extracts using known procedures to identify the plant phytochemicals namely alkaloids, carbohydrates, proteins, phenolic compounds and gums.

(A) Test for alkaloids

Solvent free 50 mg extract was stirred with few ml of dilute HCl and filtered. The filtrate was tested carefully with various alkaloid reagents as follows.

(1) Wagner’s test. To a few ml of filtrate, few drops of Wagner’s reagent were added along the sides of the test tube. A reddish – brown precipitate confirmed the test.

(2) Hager’s test. To a few ml of filtrate 1 or 2 ml of Hager’s reagent were added. A prominent yellow precipitate indicated the test as a positive.

(B) Test for Carbohydrates and Glycosides

(1) Molish test. To 2ml of filtrate two drops of alcoholic solution of α-naphthol were added, the mixture was shaken well and 1ml of concentrated sulphuric acid was added slowly along the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.

(2) Fehling test. One ml of filtrate was boiled on water bath with 1ml each of Fehling solution A and B. A red precipitate indicates the presence of sugar.

(3) Barfoed test. To 1ml filtrate, 1ml of Barfoed reagent was added and heated on a boiling water bath for 2 min. Red precipitate indicates the presence of sugar.

(4) Benedict’s test. To a 0.5 ml of filtrate, 0.5 ml of Benedict’s reagent was added. The mixture was heated on a boiling water bath for 2 min. A characteristic colored precipitate indicates the presence of sugar.

(C) Test for proteins and Amino Acids

(1) Biuret test. An aliquot of 2 ml of filtrate was treated with one drop of 2% copper sulphate solution. To this 1 ml of ethanol (90%) was added, fallowed by excess of potassium hydroxide pellets. Pink colour in ethanol layer indicated the presence of proteins.

(2) Ninhydrin test. Two drops of ninhydrin solution were added to 1 ml of aqueous filtrate. A characteristic purple colour indicated the presence of amino acids.

(D) Test for Fixed Oils and Fats

(1) Spot test. A small quantity of extract was pressed between two filter papers, oil stain on the filter paper indicated the presence of fixed oil.

(E) Test for Phenolic compounds and Tannins

(1) Ferric chloride test. The extract (500 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of Phenolic compounds.

(2) Gelatin test. The extract (50 mg) was dissolved in 5 ml of distilled water and 2 ml of 1% solution gelatin containing 10% sodium chloride was added to it. White precipitate indicated the presence of phenolic compound.

(F) Test for flavonoids

(1) Alkaline reagent test. An aqueous solution of extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

Table 1: Results of phytochemical analysis of extract

<table>
<thead>
<tr>
<th>Phytochemical tests</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid Test</td>
<td></td>
</tr>
<tr>
<td>Hager’s Test</td>
<td>+ve</td>
</tr>
<tr>
<td>Wagner’s test</td>
<td>-ve</td>
</tr>
<tr>
<td>Carbohydrate Test</td>
<td></td>
</tr>
<tr>
<td>Molish Test</td>
<td>+ve</td>
</tr>
<tr>
<td>Fehling Test</td>
<td>-ve</td>
</tr>
<tr>
<td>Proteins and Amino Acids</td>
<td></td>
</tr>
<tr>
<td>Biuret Test</td>
<td>-ve</td>
</tr>
<tr>
<td>Ninhydrin Test</td>
<td>+ve</td>
</tr>
<tr>
<td>Fixed oils and Fats</td>
<td></td>
</tr>
<tr>
<td>Spot Test</td>
<td>+ve</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td></td>
</tr>
<tr>
<td>Ferric chloride test</td>
<td>+ve</td>
</tr>
<tr>
<td>Gelatin Test</td>
<td>-ve</td>
</tr>
<tr>
<td>Flavonoid Test</td>
<td></td>
</tr>
<tr>
<td>Alkaline reagent Test</td>
<td>+ve</td>
</tr>
</tbody>
</table>

Extraction of oil

100 gm of powdered clove buds were weighed and extracted for 24hrs, in a Soxhlet apparatus using petroleum ether. The residue was again extracted using 70% (v/v) ethanol. Extract was collected and concentrated up to dryness with the help of water bath.
Isolation of oil

The extracted material was dissolved in 25 ml of petroleum ether, and 30 ml of 5% NaOH was added to the ether solution. The solution was shaken gently in separating funnel to mix up the two immiscible phases. The aqueous layer was separated and collected in a 100ml beaker, and the organic layer was transferred back to the separating funnel.

The ethereal solution was extracted twice more with fresh 25 ml portions of 5% NaOH solution. The combined aqueous solution was poured back in to the separating funnel and washed with fresh 15ml portion of petroleum ether. Then the aqueous solution was transferred to a 250ml beaker and slowly acidified with 5% HCL. The acidified solution was transferred to separating funnel and extracted with 25ml of fresh ether. Ether solution was separated and collected in a beaker and the aqueous layer was again extracted twice with fresh 25ml portions of ether. Using the same separating funnel ether solution was washed with 15ml of distilled water. The ether solution was then dried over magnesium sulfate; the magnesium sulfate was removed by filtration and the ether from the filtrate by evaporation on a water bath. The percent recovery from cloves was determined and the product was analyzed by Gas Chromatography and Mass Spectroscopy (GC-MS).

Chemical analysis

Isolated sample was analyzed on a Shimadzu QP 5050a Gas chromatograph interfaced to a Mass spectrometer (GC/MS) instrument employing the following conditions: fused silica capillary column (cbp-5; 30m X 0.25µm x 0.25µm) which was programmed as follows: 60°c for 2 min and then up to 240°c at 3°c/min, then to 270°c at 10°c/min ending with a 10 min at 270°c. The carrier gas was He at a flow rate of 1ml/min, split mode, with ratio of 1:5, and injection volume of 1µl in CH₂Cl₂ and the ionization voltage, 70ev.

RESULTS AND DISCUSSION

Phytochemical Analysis

The data sheet for various types of phytochemicals which were found in Syzygium aromaticum is as follows (Table 1).

Identification of extracted product

Sensory evaluation: Separated oil was partially identified as clove oil with following observations:

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Appearance</td>
<td>Slightly murky</td>
</tr>
<tr>
<td>Odour</td>
<td>Cloves like(aromatic)</td>
</tr>
<tr>
<td>Intensity of odour</td>
<td>Strong, pungent</td>
</tr>
</tbody>
</table>

Analytical evaluation

In gas chromatographic analysis a compound was reported to have retention time of 7.93 minutes, whereas the mass spectroscopic analysis showed 164 as m/z value, which was in accordance with the molecular weight of eugenol (include reference for this).

From above analysis the isolated compound was supposed to be eugenol. The chromatographs obtained from GC/MS analysis are shown below Figure 1 &2:

Figure 1: Gas chromatogram of isolated compound

Figure 2: Mass spectrum of isolated compound

CONCLUSION

Cloves have historically been used as carminative, as local anesthetic and natural anthelminthic, anti-pyretic agent with the main active constituent, “eugenol”, comprising 72-90% of total active constituents. Active
constituents of clove buds were extracted by Soxhlet extraction using 70% v/v methanol. On the basis of mass and gas chromatographic data, isolated product was characterized as eugenol, with a retention time of 7.93 min. and a molecular weight (m/z) of 164. Total 7.70 g of eugenol was recovered from 100 g of cloves. This corresponds to a percent recovery of 7.70%.

REFERENCES


Singh AK, Dhamanigi SS, Asad M. Common Fragrance and Favor Materials. Indian J Pharmacol 2009; 41: 28-33