

QUALITATIVE PHYTOCHEMICAL ANALYSIS AND ESSENTIAL OIL EXTRACTION OF *Pimenta dioica* AND IT'S ANTIBACTERIAL ACTIVITY

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ABSTRACT

Pimenta dioica (L.) Merr commonly known as allspice is an industrially and therapeutically important, evergreen aromatic spice plant widely used in food, perfumery and cosmetic industries around the globe. The extraction of essential oils of *Pimenta dioica* was carried out by the hydrodistillation method using Clevenger apparatus. Preliminary phytochemical screening shows the presence of volatile oil, starch, proteins, tannins, saponins, fats, alkaloids and glycosides etc. Phytochemicals like steroids, alkaloids, phenolics, flavonoids, tannins, glycosides and essential oil isolated from the plant has medicinal and pharmaceutical importance. Essential oils are the most important compounds produced during secondary metabolism in aromatic plants. The inhibitory effect of leaf extracts was determined by disc diffusion method against clinical isolates of *Staphylococcus aureus* and *Escherichia coli*. The plant can be potential candidate for the development of agents active against pathogenic bacteria.

KEYWORDS: *Pimenta dioica* , Phytochemistry, Essential Oil, Secondary Metabolites, Antibacterial Activity, Disc Diffusion Technique

Pimenta dioica (L.) Merill. and it belongs to the family Myrtaceae is well known for it' s characteristic aroma, therapeutic& culinary qualities . Which resemble to aroma and flavour of Clove, Nutmeg & cinnamon and hence it is called Allspice. Phytochemical constituents present in this plants are phenyl propanoids and glycosides (Kikuizaki *et al.*,1999) tannins (Marzouk *et al.*,2007).

Essential oils are the volatile, odoriferous oils obtained from plant. Essential oils from aromatic plants possess a broad range of biological activities, being antimicrobial one of its main medical properties reported (Viuda-Martos *et al.*,2008). Essential oils are part of plant metabolites and they are usually composed of volatile terpenes. These compounds can be located indifferent part of the plant. The constitute 0.1% to 1% of plant dry weight (Lopez, 2004).

Chemical constituents in the plant are responsible for their medicinal as well as their toxic properties. Plant constituents represent an important source for antibacterial legends as tannins, flavonoids and volatile oil. Tannins are water soluble polyphenolic compounds with high molecular weight. They have the ability to form complexes with proteins (Ozidal *et al.*,2013).

Pimenta dioica extract was found to inhibit *S. aureus* and *P. aeruginosa* (Marzouk *et al.*, 2007). The essential oil of berries was found to inhibit *Pseudomonas putida*, *E. coli*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Staphylococcus aureus* (Oussalah *et al.*, 2006).

Pimenta oil can be helpful for the digestive system, for cramp, flatulence, indigestion ,nausea, depression, nervous exhaustion, tension, neuralgia and stress and is used us natural repellent .The essential *P. dioica* leaf and fruit oil is also used in perfumes, after shaves and commercial food flavouring (Sharma, 2003).

MATERIALS AND METHODS

Plant Material



P. dioica belongs to the family Myrtaceae . This is a tropical, evergreen plant which grows up to 15cm tall with a pale brown bark, twigs flattened. The plant *P.dioica* were collected from karukachal, kottayam. The identification of the plant material were confirmed by gamble.

ESSENTIAL OIL EXTRACTION

The volatile oils of *P. dioica* leaves were obtained by hydrodistillation in Clevenger apparatus. In this method, the sample is completely immersed in water and the still is brought to the boil. After 3hrs of continuous extraction of oil was separated and collected in glass vials and stored in refrigerator.

PHYTOCHEMICAL SCREENING

The sample of *P. dioica* were washed with water and sliced into for 10 days and pulverized to powder using mixing. The powdered leaves were cold extracted with 50ml of 95% ethanol for 3 days. The extract obtained were filtered using Whatman filter paper 1 and concentrated to powdered form by complete evaporation of the extraction solvent. The extract were then subjected to various qualitative tests using standard methods, to determine the presence or absence of various phytoconstituents such as alkaloid, flavonoid, tannins, saponins, terpenoids etc.

ANTIBACTERIAL ACTIVITY

The antibacterial activity of essential oil from the plant parts of *Pimenta dioica* were screened against two bacterial strains, gram positive (*S. aureus*) & gram negative (*E. coli*). Petriplate containing 10ml Muller Hinton medium were seeded with 24hrs culture of bacterial strain. Well were cut and 25µl of the given sample (of different concentrations) were added. The plates are then incubated at 37°C for 3-4hrs. The diameter of incubation zone formed around the well was then measured.

RESULTS AND DISCUSSION

Essential Oil Extraction

Approximately 0.3-0.5ml essential oil is extracted by hydrodistillation method using Clevenger apparatus. The extraction is fast at beginning but get slow gradually. When the maximum amount of extractable oil is obtained, the oil yield level remains constant even by extending the extraction time.

Phytochemical Screening

Preliminary phytochemical screening from ethanolic extract of *Pimenta dioica* was carried out using various chemical assays in order to identify either the presence or absence of secondary metabolites such as alkaloid, flavonoid, saponin, tannin, steroid etc. The result summarized in (Table 1).

Table 1:

Sl. No.	Phytoconstituents	Intensity
1	Alkaloid	+
2	Flavonoid	+++
3	Phenol	+
4	Saponin	+
5	Tannin	++
6	Protein	+
7	Steroid	+++

Key: +=present; ++=present in moderate level; +++=present in high level; -=absent

Appearance of orange colour indicates the presence of alkaloids. The pharmacological significances are reported by various workers. It possess antidiarrhoeal (Kumar *et al.*, 2010).

Pink/red colour indicates the presence of flavonoids. It forms complex with cell wall, binds to adhesins and showed antimicrobial activity (Cowan *et al.*, 1999).

Saponins present in extract of leaf namely ethanol. Saponins lead to vacuolization and disintegration of ligaments through anthelmintic activity.

Blue, green or brownish green colour indicates the presence of Tanins. Tannins are antimicrobial agents that could inhibit the growth of microorganisms (Trease and Evans, 2002).

Proteins are present in the ethanolic extract of pimenta dioica. Proteins are Bioactive against certain ailments.

Antibacterial Activity

The antibacterial activity of essential oil from the plant parts of pimenta dioica were screened against two bacterial strains such as *S. aureus* and *E. coli*. The antibacterial potential of essential oil were measured in terms of zone of inhibition of bacterial growth. The result of the present study are shown in (Table2).

The bacteria assayed include both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) strains. The essential oil of produced good results against both Gram positive bacteria (*S.aureus*) and Gram negative bacteria *E. coli*.

Table 2:

CULTURE	A	C	B
<i>E. coli</i>	1.1	1.7	0
	1.2	1.6	0
	1.1	1.7	0
	1.1	1.6	0
	1.2	1.6	0
	1.2	1.6	0
<i>S. aureus</i>	2.4	2.3	0
	2.4	2.3	0
	2.4	2.2	0
	2.3	2.2	0
	2.4	2.3	0
	2.3	2.3	0

CONCLUSION

Essential oil extracted by using cleverger apparatus. The oil have allspice smell. The extract of *Pimenta dioica* plant leaves expressed positive towards secondary metabolites such as alkaloids, flavonoids, phenols, tannins, steroid, phenol, saponin and proteins.

In this work the Antibacterial activity of essential oil is studied and it is revealed that that the antibacterial potential of essential oil is varied for gram negative and gram positive bacteria. The essential oil shows more activity against gram positive bacteria. The observed inhibitory activity could be attributed to the presence of bioactive secondary metabolites.

In conclusion the present study demonstrates that the ethanolic extract of *Pimenta dioica* contains pharmacologically active substances, The preliminary phytochemical screening revealed that the presence of secondary metabolites in the ethanolic extract. This could be crucial step in further studies on the phytochemical, biological. Structure-function relationship of *Pimenta dioica* which is already reported to be of therapeutic importance.

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