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Investigation of Bioactive Compounds and Their Effectiveness in Antimicrobial Activity of Ornamental Plant *Dyanthus Caryophyllus*

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ABSTRACT
Plants are a source of phytomedicines. Amongst the traditional herbs used as phytomedicines in Kisii region, southwest Kenya are *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica.* Herbal medicines have been used for thousands of years. The practice continues today because of its biomedical benefits and place in cultural believes in many parts of the world.The economic reality of the inaccessibility of modern medications for many societies has also played a major role in the broad use of herbal medicines. The World Health Organization has recognized the contribution and value of the herbal medicines used by a large segment of world's population. A study was carried out on *Dianthus caryophyllus*, an Indian herb used for various ailments by traditional healers. Present study carried out phytochemical analysis and antimicrobial investigation of different solvent and aqueous extracts of the flower and leaves against a panel of clinically significant bacterial and fungal strains. Phytochemical studies revealed the presence of carbohydrates, saponin, amino acid, chloride, phenolic compounds, tannins, flavonoids, alkaloid, proteins and steroids. Susceptibility testing by disc diffusion assay revealed significant antimicrobial activity. The samples were extracted with five solvents namely aqueous, dmso, chloroform, acetone and ethanol. Portions of the crude extracts were screened against all the tested organisms, ethanol extracts of flower exhibited appreciable activity against gram positive bacteria *Staph. aureus* (20mm) and fungus *Aspergillus fumigatus*(15mm). Likewise, chloroform extracts of leaf showed higher activity on the gram positive bacteria *Staph. aureus* (13mm) and fungus *Penicillium* sp (12mm).Thin Layer Chromatography was carried, two spots were observed in each leaf and stem sample. The sample was further studied by FT-IR, it shown 19 and 16 Functional groups/compounds between the spectra 400-4000nm in flower and leaf respectively. The study findings provide supportive evidence for the use of *Dianthus caryophyllus* in traditional medicines.

KEYWORDS: Antibacterial activity, *Dianthus caryophyllus*, Medicinal plants, Agar Dilution, Alkaloids.

INTRODUCTION

Plants are part and parcel of human society to combat diseases from the dawn of civilization. The use of plants as medicine is widespread throughout the world. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment.. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%.. Medicinal plants and their derivatives are thus looked upon not only as a source of affordable healthcare but also as an important commodity item of international trade and commerce.

Historically, plants have provided a source of inspiration for novel drug compounds, as plant-derived medicines have

made large contributions to human health and well – being. A major part of the total population in developing countries still uses traditional folk medicines obtained from plant resources (Srivastava*et al*., 1996). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Plants produce a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry and it is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens (Shokeen*et al*., 2009). Plant based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials of plant origin have enormous therapeutic potential (Salau and Odeleye,2007).Medicinal plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals.. The extraction and characterization of active compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic value (Asakawa, 2007). Medicinal plants are rich sources of antimicrobial agents (IsmetAra*et al* ., 2012). They are the sources of natural pesticides that make excellent leads for new pesticide development (Satish*et al*., 2008).. The biological activities of plants may be due to the presence of a diverse group of chemical compounds including steroids, glycosides, phenolics, glycosides, anthocyanins, flavonoids etc. (Quideau*et al*., 2011). The search of biologically active compounds from plants has always been of great interest to scientists looking for new sources of useful drugs against infectious diseases.

Dianthus caryophyllus, **carnation** or **clove pink**, is a species of *Dianthus*. It is probably native to the Mediterranean region but its exact range is unknown due to extensive cultivation for the last 2,000 years. It is a herbaceous perennial plant growing to 80 cm tall. The leaves are glaucous greyish green to blue-green, slender, up to 15 cm long. The flowers are produced singly or up to five together in a cyme; they are 3–5 cm diameter, and sweetly scented; the original natural flower colour is bright pinkish-purple, but cultivars of other colours, including red, white, yellow and green, have been developed.Plant kingdom is rich in wide variety of free radical scavenging molecules, such as phenolic compounds (e.g.phenolic acids, flavonoids, quinines, coumarins, lignin's, stilbenes, tannins), nitrogen containing compounds (alkaloids and amines), vitamins, terpenoids (including carotenoids) and some other endogenous metabolites, which are rich in antioxidant activity (Zheng.,2001).Phytochemicals are present in the various parts of the plants are of great significance in therapeutic treatments. The biological activities of plants may be due to the presence of a diverse group of chemical compounds including steroids, glycosides, phenolics, glyosides, anthocyanins, flavonoids etc.(Owolabi*et al* ., 2007).

MATERIALS AND METHOD

Dyanthus caryophyllus TAMIL – HEAVENLY FLOWER

Common names: Carnation, Divine flower, Clove pink, Gilly Flower .

Dianthus caryophyllus, carnation or clove pink, is a species of *Dianthus*. It is probably native to the Mediterranean region but its exact range is unknown due to extensive cultivation for the last 2,000 years. It is a herbaceous perennial plant growing to 80 cm tall. The leaves are glaucous greyish green to blue-green, slender, up to 15 cm long. The flowers are produced singly or up to five together in a cyme; they are 3–5 cm diameter, and sweetly scented; the original natural flower colour is bright pinkish-purple, but cultivars of other colours, including red, white, yellow and green, have been developed.

Plant material of *Dianthus caryophyllus*was collected from Marthandam, Kanyakumari District. The flower part used for this study were rinsed severally with clean tap water to make it dust and debris free and subjected to drying in a dark place at room temperature for few days (upto the leaf get shade dry). The dried parts were ground in electric chopper to get fine powder form for further use.

Preparation of Extracts

The prepared powder of *Dianthus caryophyllus* (leaf & stem) was subjected to soxhlet extraction using aqueous, Acetone, Dimethyl sulfoxide, Choloroform and ethanol. Each 5grams of dried, powder of plant material was filled separately in the thimble and extracted successively with 60ml of solvents using a soxhlet extractor for 3hours. After solvent evaporation, each of these solvent extract was weighted was weighted in room temperature until further use.

Qualitative analysis of phytochemical constituents

All the extracts were subjected to systematic phytochemical screening for testing for the presence of chemical constituents (Sofowra, 1993; Harborne, 1973).

Tests for Carbohydrates (Benedict's test)

Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Tests for Amino Acids (Ninhydrin test)

For the analysis of amino acid 3 ml test solution and 3 drops 5% Ninhydrin solution were heated in water bath for 10 min. Observed for purple or bluish colour, the appearance of colour indicates the presence of amino acids.

Tests for Proteins (Biuret test)

3 ml of each test solution was added to 4% NaOH and few drops of 1% $CuSO_4$ solution into separate tubes. The tubes were observed for violet or pink colour formation.

Tests for Vitamin C

1 ml of 2% w/v solution was diluted with 5ml of water. 1 drop of freshly prepared 5% w/v solution of sodium nitroprusside and 2 ml of diluted sodium hydroxide solution were added. Then 0.6 ml of hydrochloric acid was added dropwise and stir, the yellow colour turns blue it's indicates positive results.

Tests for Chloride

3 ml test solution prepared in HNO_3 and few drops 10% $AgNO_3$ solution was added. White precipitate of $AgCl_2$ is observed.

Tests for Tannins

With 2-3 ml test solution, 5% $FeCl_3$ solution was added and observed for deep blue-black colour reactions.

Tests for Alkaloids (Wagner's test)

2-3 ml filtrate was taken into separate tubes. To that few drops of Wagner's reagent was added and observed reddish brown precipitate.

Detection of flavonoids

Lead acetate Test: The extracts were treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids.

Test for phlobatannins

Formation of red precipitate when fruit sample was boiled with 1% aqueous hydrochloric acid indicates the presence of phlobatannins.

Tests for Steroids (Salkowski Reaction)

To 2 ml of sample, 2 ml chloroform and 2 ml Concentrated H_2SO_4 were added and observed chloroform layer for red colour and acid layer for fluorescence.

Tests for Phenolic compounds (Ferric chloride test)

The extract was diluted to 5 ml with distilled water. To that a few drop of neutral 5% ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds.

Test for saponins

The samples were diluted with distilled water and made into 20 ml. The suspension was shaken well in graduvated cylinder for 15 minutes; 2 cm layer of foam indicates the presence of saponins.

Anti-microbial activity assay of crude extracts

Anti-microbial activities of aqueous, Dimethyl sulfoxide, acetone, chloroform and ethanol extract were determined by well diffusion method (Anushia*et al.,* 2009). Four bacterial strains (namely *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus* and *Staphyloccocusaureus*) and three fungal strains (namely *Aspergilusniger*, *Aspergillusfumigatus*and *Penicillium*sp) were used in this investigation. The media used for antibacterial test were Nutrient Broth. The test bacterial strains were inoculated into nutrient broth and incubated at 37^{o}C for 24hrs. After the incubation period, the culture tubes were compared with the turbidity standard. Fungal inoculums were prepared by suspending the spores of fungus (as previously cultured) in saline water mixed thoroughly, made turbidity standard and used. Bioassay was carried out by Agar well diffusion method. Fresh bacterial culture of 0.1 ml having 108 CFU was spread on nutrient agar (NA) plate using swab. The fungal strains also the same but the medium was Potato dextrose agar (PDA). Wells of 6 mm diameter were punched off into medium with sterile cork borer and filled with 50µl of plant extracts by using micro pipette in each well in aseptic condition. Plates were then kept in a refrigerator to allow pre-diffusion of extract for 30 minutes. Further the plates were incubated in an incubator at 37^{o}C for 24hours and 28-30^{o}C for 3-4 days for bacterial and fungal cultures respectively. The antimicrobial activity was evaluated by measuring the zone of inhibition.

Thin Layer Chromatography

The slurry of silica gel G prepared with glass distilled water in the ratio 1:2 (w/v) was poured on glass slide with a layer of silica gel in 500 µm thickness. The coated plates were activated at 80^{o}C for 3 hours (Anushia*et al.*, 2009). In that study, n-Butanol, Acetic acid and methanol (2:2:1) was used as solvent. The concentrated plant extract of 0.5 mg was loaded on the TLC plates just above 2 cm from the bottom using a capillary tube. The plates were reserved in a developing jar containing the solvent mixture. The plates were removed and allowed at room temperature for 30 minutes and results were observed by UV radiation.

Rf = Distance of spot from origin / Distance travelled by solvent

Analysis of plant extracts by Infrared Spectrophotometer (FTIR)

ATR model FTIR Spectrophotometer (Bruker Co., Germany) was used for the analysis of the plant material of *Dianthus caryophyllus.* The spectrum (400-4000nm) was recorded using Attenuated Total Reflectance (ATR) technique beach measurement.

RESULTS AND DISCUSSION

Extraction was carries out Soxhlet apparatus aqueous, acetone, DMSO, chloroform and ethanol as extracting solvents. The resulting extracts were filtered using filter paper and each filtrate was concentrated with a rotary evaporator. All the extracts were kept in desiccators at 4^{o}C until use.

Qualitative analysis phytochemical constituents

All the extracts were subjected to systematic phytochemical screening for testing for the presence of chemical constituents. The results of phytochemical screening of leaf and

stem parts of *Dianthus caryophyllus*were mentioned in the following table (Table 1). In *Dianthus caryophyllus*flower, the aqueous extract showed positive results for carbohydrate, chloride, alkaloids, flavanoids and steroids; DMSO extract showed positive results only for flavanoids and steroid; acetone extract showed positive results for carbohydrate, aminoacid, tannin and flavanoid; chloroform extract showed positive results for carbohydrate, tannin and steroid; ethanol extract showed positive results for carbohydrates, chloride, tannin and phenolic compounds.

Table 1: Qualitative analysis of phytochemical constituents

SL.No.	Chemical Constituents	*Dianthus caryophyllus* flower extracts					*Dianthus caryophyllus* leaf extracts				
		Aqueous	DMSO	Acetone	Chloroform	Ethanol	Aqueous	DMSO	Acetone	Chloroform	Ethanol
1	Carbohydrates	+	-	+	+	+	+	_	_	_	+
2	Protein	-	-	-	-	-	-	-	-	-	-
3	Amino acid	-	-	+	-	-	-	-	+	-	-
4	Vitamin C	-	-	-	-	-	-	-	-	-	-
5	Chloride	+	-	-	-	+	+	-	-	-	+
6	Tannins	-	-	+	+	+	-	-	+	+	+
7	Alkaloids	+	-	-	-	-	+	-	-	-	-
8	Flavonoids	+	+	+	-	-	+	+	+	-	-
9	Phlobatannins	-	-	-	-	-	-	-	-	-	-
10	Steroids	+	+	-	+	-	+	+	-	+	-
11	Phenolic Compounds	-	-	-	-	+	-	-	-	-	+
12	Saponins	-	-	-	-	-	-	-	-	-	-

'+' presence of compound; '-' absence of compound

Anti-microbial activity assay of crude extracts

Antimicrobial activity of aqueous, acetone, DMSO, chloroform and ethanol extracts were determined by well diffusion method using the test bacterial (*Escherichia coli, Klebsiella pneumonia, Bacillus subtilis,* and*Staphyloccocusaureus*) and fungal cultures (*Aspergillusniger, Asperillus fumigates* and*Penicilliumsp*). After incubation the plates were observed for zone of inhibition and the antimicrobial activity was evaluated by measuring the zone of inhibition (Table 2). Leaf of aqueous extract showed activity on *K. pneumoniaee* (14mm) only, and no activity on other test organisms; DMSO extract showed activity on K.pneumonia (10mm),*Staph. aureus*(11mm), *Bacillus cereus* (10mm), *Aspergillus fumigatus*(14mm), *Aspergillus niger*(10mm), *Penicillium sp*(14mm) and no activity on *Escherichia coli*; Acetone Extract showed activity on *Escherichia coli* (11mm), *K.pneumonia*(10mm), *Staph. aureus*(12mm), *Aspergillus fumigatus*(10mm), *Aspergillus niger*(13mm), *Penicillium sp*(13mm), and no activity on *Bacillus cereus*; Chloroform extract showed activity on *Escherichia coli* (10mm), *Staph. aureus*(16mm), *Bacillus cereus* (11mm), *Aspergillus fumigatus*(12mm), *Aspergillus niger*(13mm) and no activity on *K. pneumoniaee* and *Penicillium* sp; Ethanol extract showed activity on *Escherichia coli* (10mm), *Staph. aureus*(20mm), *Bacillus cereus* (12mm), *Aspergillus fumigatus*(15mm), *Aspergillus niger*(12mm), *Penicillium sp*(10mm) and no activity on *K. pneumoniaee*.

Table 2: Anti-microbial activities of *Dianthus caryophyllus*flower and leaf extracts

SL. No.	Test Organisms	*Dianthus caryophyllus*flower extracts					*Dianthus caryophyllus*leaf extracts				
		Aqueous	DMSO	Acetone	Chloroform	Ethanol	Aqueous	DMSO	Acetone	Chloroform	Ethanol
Bacteria											
1	*Escherichia Coli*	-	-	11	10	10	10	-	12	10	11
2	*K.pneumoniae*	14	10	10	-	-	10	11	11	11	10
3	*Staph.aureus*	-	11	12	14	20	-	10	10	13	11
4	*Bacillus cereus*	-	10	-	11	12	-	10	10	10	14
Fungi											
1	*Aspergillus fumigates*	-	14	10	12	15	-	11	10	10	10
2	*Aspergilllus Niger*	-	10	13	13	12	-	10	10	11	11
3	*Penicillium sp.*	-	14	13	-	10	-	10	-	10	12

Stem of aqueous extract showed activity on *Escherichia coli* (10mm), *K. pneumoniaee* (10mm) and no activity on other test organisms; DMSO extract showed activity on *K.Peneumoniae*(11mm), *Staph. aureus*(10mm), *Bacillus cereus* (10mm), *Aspergillus fumigatus* (11mm), *Aspergillus niger* (10mm), *Penicillium sp* (10mm) and no activity on *Escherichia coli*; Acetone Extract showed activity on *Escherichia coli* (12mm), *K. pneumoniaee* (11mm), *Staph. aureus*(10mm), *Bacillus cereus* (10mm), *Aspergillus fumigatus*(10mm), *Aspergillus niger*(10mm) and no activity on Bacillus cereus, *Penicillium* sp; Chloroform extract showed activity on *Escherichia coli* (10mm), *K. pneumoniaee* (11mm), *Staph. aureus*(13mm), *Bacillus cereus* (10mm), *Aspergillus fumigatus*(10mm), *Aspergillus niger*(11mm), *Penicillium sp*(10mm); Ethanol extract show activity on *Staph. aureus*(11mm), *Bacillus cereus* (12mm), *Aspergillus fumigatus*(10mm), *Aspergillus niger*(11mm), *Penicillium sp*(12mm).

In the above observation ethanol extracts of flower showed higher activity on the gram positive bacteria *Staph. aureus*(20mm), and fungus *Aspergillus fumigatus*(15mm). Likewise chloroform extracts of leaf showed higher activity on the gram positive bacteria *Staph. aureus*(13mm), and fungus *Penicillium sp*(12mm).The antimicrobial of activity may due to the presence of phytochemical constituents like alkaloids, flavanoids, tannins and steroids. The result from this work has revealed the medicinal potential of these plants in the treatment of bacterial and fungal diseases.

FTIR analysis

The FTIR analysis of *Dianthus caryophyllus* flower showed 19 functional group/ion and leaf showed 16 functional group/ion between 400-4000 spectra.

Fig 1: FTIR spectra of *D. caryophyllus* flower

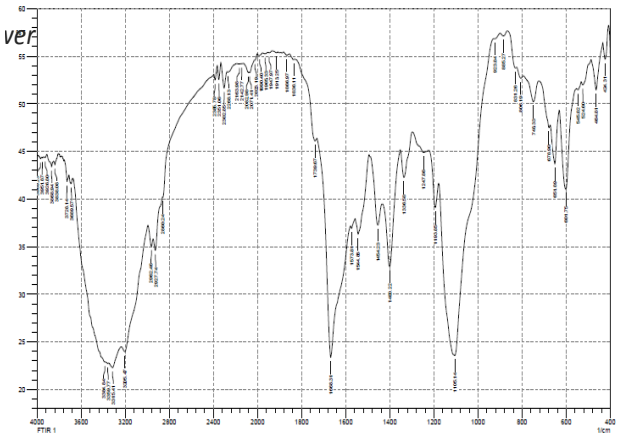
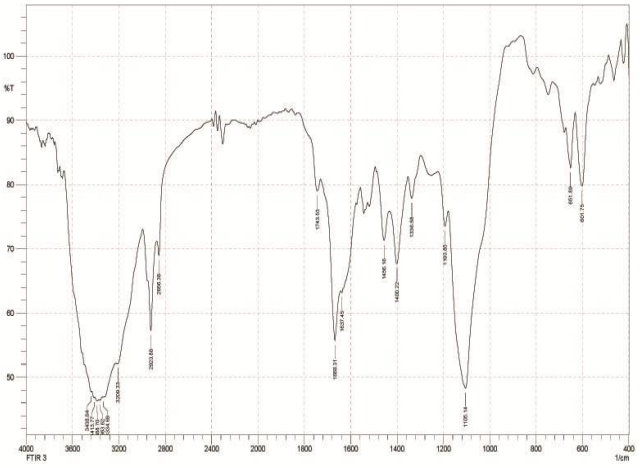


Fig 1: FTIR spectra of *D. caryophyllus* leaf



Thin Layer Chromatography

Thin layer chromatography was performed for both leaf and stem material. In this study, two spots were observed in each leaf and stem sample, the rf value was calculated using the following formula:Rf=Distance travelled by solute / Distance travelled by solvent. The flower sample showed green and pale green with the Rf value of 0.95 and 0.72 respectively, and the leaf extract exhibited pale yellow and green spots with the Rf values 0.64 and 0.68 respectively (Table 3).

Table 3: TLC analysis results of plant materials

Sample name	Color of band	Rf value
***D. caryophyllus* flower**	Green	0.95
	Pale green	0.72
***D.caryophyllus* leaf**	Pale yellow	0.64
	Pale green	0.68

Analysis of plant extracts by Infrared Spectrophotometer (FTIR)

The above chromatographic diagrams represents that the FTIR analysis of *Dianthus caryophyllus*flower and leaf extracts respectively. There are 19functional group / ion in flower extract and 16 functional groups /ion in leaf extract were detected between 400-4000 nm spectra

CONCLUSION

The plant of *Dianthus caryophyllus*was collected from Marthandam, Kanyakumari District. The dried samples of leaf and stem were ground in electric chopper to get fine powder form for further use. Powdered plant material was filled separately in the thimble and extracted successively using a soxhlet extractor with water, actone, DMSO, chloroform and Ethanol. All the extracts were subjected to phytochemical screening for the presence of chemical constituents. The results revealed the presence phytochemical constituents like carbohydrates, amino acids, chloride, tannins, alkaloids, flavanoids, phenolic compounds and steroids. Antimicrobial activities of all the extract were determined by well diffusion method. They showed good activity against all the tested organisms, ethanol extracts of flower showed higher activity on the gram positive bacteria *Staph. aureus*(20mm) and fungus *Aspergillus fumigatus*(15mm). Likewise, chloroform extracts of leaf showed higher activity on the gram positive bacteria *Staph. aureus*(13mm) and fungus *Penicillium sp*(12mm).Thin Layer Chromatography was carried, two spots were observed in each leaf and stem sample. The sample was further studied by FT-IR, it shown 19 and 16 Functional groups/compounds between the spectra 400-4000nm in flower and leaf respectively.

There are fewer reports were available for study about *Dianthus caryophyllus* as an antimicrobial agents. This study supports the

medicinal use of *Dianthus caryophyllus* for the treatment of various infectious diseases in different regions of the world. More work is needed to isolate the bioactive components in the plant.

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