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In vitro studies on antibacterial activity and separation of active compounds of selected flower extracts by HPTLC

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ABSTRACT

The antimicrobial activity of methanol extract of Nerium indicum, Tagetus erecta, Chrysanthemum leucanthemum, Rosa centrifolia, Jasminum angustifolium, Torenia fournieri against Gram negative and Gram positive bacteria were studied invitro. The objective of this research was to confirm the antibacterial activity and perform HPTLC analysis of methanolic extracts of various flower extracts. The methanolic extracts of Nerium indicum, Tagetus erecta, Rosa centrifolia, Torenia fournieri exhibited growth inhibition on selected bacterial strains viz., Bacillus sp., Escherichia coli., Klebsiella sp., Yersinia sp., Enterococcus sp. Based on the results, the methanol extract of Rosa centrifolia was considered to be the most effective and also indicated that all the flower extracts exhibited inhibitory action against the growth of Lactobacillus sp., whereas Chrysanthemum leucanthemum, Jasminum angustifolium, did not show any inhibition on the test bacterial species. Further the extracts were separated on the TLC plates on selected mobile phase and analysed in HPTLC using CAMAG software.

Keywords: *Nerium indicum, Tagetus erecta, Chrysanthemum leucanthemum, Rosa centrifolia, Jasminum angustifolium, Torenia fournieri*, antibacterial activity, agar well diffusion method, HPTLC.

INTRODUCTION

Traditional medicines are wide spread throughout the world. Only 1% of angiospermic plants have been scientifically evaluated for their medicinal value [1]. Plants have a great potential for producing new drugs of great benefit to mankind [2]. The prevailing threat on existing plant wealth compels us for an immediate scientific evaluation of the medicinal properties of these

plants and globally there has been an increased interest to identify compounds that are pharmacologically potent that have low or no side effects for use in therapeutic purpose. Recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine [3]. Plant-based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials needs to occur. Secondary plant metabolities (Phytochemicals), with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [4]. The present study deals with antibacterial activity of the flower extract viz., *Nerium indicum, Tagetus erecta, Chrysanthemum leucanthemum, Rosa centrifolia, Jasminum angustifolium, Torenia fournieri*. The main objective of this study is to search for the active fraction with a strong antimicrobial activity which could serve for the development of new antimicrobial agents. In order to access their antibacterial activity, the methanolic flower extracts were assayed using agar well diffusion method. The development of resistant strains of bacteria has increased the need for new antibiotics [5]. Further HPTLC analysis provides scientific basis for the use of the flower extract in development as antibacterial and anti-inflammatory agent.

EXPERIMENTAL SECTION

Plants Used:

The flowers of *Nerium indicum, Tagetus erecta, Chrysanthemum leucanthemum, Rosa centrifolia, Jasminum angustifolium, Torenia fournieri* were used for the study. These plants were cultivated for its flowers.

Micro organisms used:

The cultures of *Bacillus sp., Escherichia coli, Staphylococcus sp., Klebsiella pneumoniae, Lactobacillus sp., Yersinia sp., Enterococcus sp. and Pseudomonas sp.* were collected from ATCC. The bacterial stock cultures were maintained at 4ºC.

Preparation of Methanolic extract:

Flowers of *Nerium indicum, Tagetus erecta, Chrysanthemum leucanthemum, Rosa centrifolia, Jasminum angustifolium, Torenia fournieri* were shade dried ground and extracted using methanol. The extract was collected and kept in a shaker for 24 hrs at room temperature. Later using a water bath evaporator, the solvent was made to evaporate and the residual extracts were weighed and stored. The compounds in the extracts obtained were completely dissolved in 70% ethanol. Stock was prepared from appropriate amount and spotted on the nutrient medium to get a final concentration of 500 and 1000 ppm. These two concentrations were used in the bacterial growth inhibition assay.

Antibacterial assay:

Antibacterial effect of the selected flower extracts were attempted by agar well diffusion assay. The plates containing sterile Nutrient agar (pH 7.4) were swabbed with bacterial inoculums grown in nutrient broth (pH 7.2) overnight. The plates containing agar medium were bored with a sterile well cutter. About 300 µl (1000ppm) and 150µl (500ppm) of the respective flower extract was poured into the well. The extract containing the plates were incubated at 37ºC overnight and examined for zone of inhibition to identify the antibacterial activity of the flower extracts.

Separation of active compounds by HPTLC:

The selected flower extracts were dissolved in methanol and the samples were separated on Thin layer Chromatographic plates using various solvent systems. It is necessary for separation, purification and characterization of biologically active compounds using chromatographic techniques for the synthesis of novel antibiotics. Mobile phase was decided based on the separation of compounds on the plates. Butanol: water: acetic acid: formic acid 9:0.5:0.25:0.25 was selected for separation for *Nerium indicum, Tagetus erecta, Rosa centrifolia* extracts whereas Ethyl acetate: water: acetic acid 9:0.5:0.5 was selected for *Torenia fournieri* extract. Samples were loaded on the Thin layer Chromatographic plates and taken for HPTLC processing. Results were observed in HPTLC monitor and analyzed using CAMAG software.

RESULTS

Antibacterial assay:

The antibacterial activity of methanol flower extract against the tested eight bacterial strains is presented in **Table 1.** The methanol extracts of the selected flowers exhibit antibiogram activity against the tested bacteriological cultures at 500ppm and 1000ppm concentration of the flower extracts comparatively, whereas all the factors showed higher inhibition at 1000ppm rather than at 500ppm.

Table 1: Antibacterial effect of various flowers extract on the test cultures as zone formation

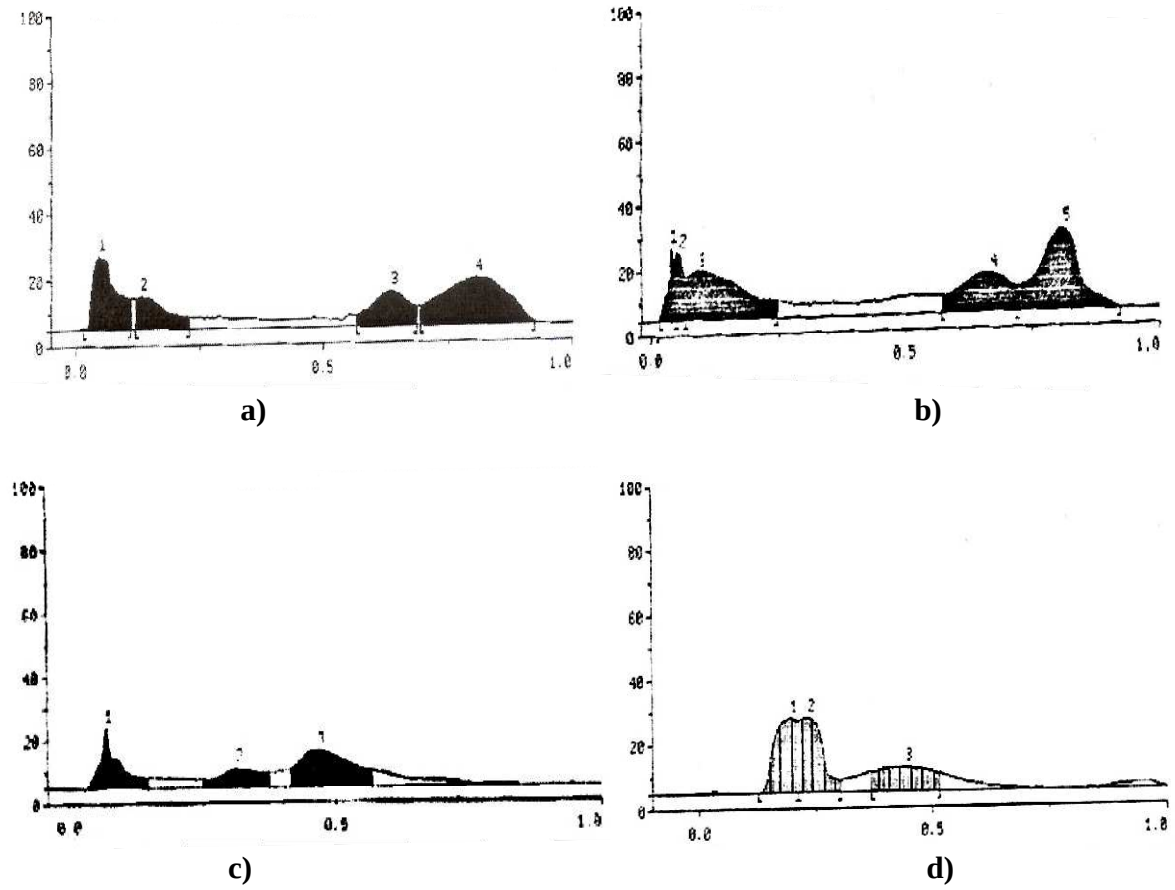
S.No.	Bacterial strains	*Nerium indicum*		*Tagetus erecta*		*Chrysanthemum leucanthemum*		*Rosa centrifolia*		*Jasminum angustifolium*		*Torenia fournieri*	
		500	1000	500	1000	500	1000	500	1000	500	1000	500	1000
1.	*Bacillus* sp.	+	0.1	0.2	0.25	-	-	0.35	0.5	-	-	0.1	0.2
2.	*Escherichia coli*	-	-	0.2	0.35	-	-	0.3	0.5	-	-	0.05	0.1
3.	*Staphylococcus* sp.	+	0.1	0.2	0.35	-	-	0.2	0.4	-	-	0.05	0.1
4.	*Klebsiella* sp.	-	+	0.2	0.35	-	+	0.3	0.5	-	+	0.1	0.15
5.	*Lactobacillus* sp.	0.1	0.3	0.3	0.5	0.1	0.3	0.4	0.6	0.6	0.4	0.3	0.5
6.	*Yersinia* sp.	+	0.1	0.2	0.3	-	+	0.4	0.55	0.55	+	0.2	0.3
7.	*Enterococcus* sp.	-	+	0.2	0.3	-	+	0.4	0.5	0.5	+	0.25	0.3
8.	*Pseudomonas* sp.	+	0.15	0.2	0.4		+	0.3	0.6	0.6	+	0.25	0.4

**Zone of inhibition in cm* *'+' – Positive* *'-' – Negative*

The methanol extract of *Nerium indicum* showed inhibition of *Bacillus sp., Escherichia coli, Yersinia sp., Staphylococcus sp.* at 500ppm and 1000ppm, whereas no inhibitory effect was observed against *Pseudomonas sp., Lactobacillus sp., Enterococcus sp.,* and *Klebsiella sp.* The methanol extract of *Tagetus erecta* inhibited the growth of *Bacillus sp., Escherichia coli., Yersinia sp., Klebsiella., Enterococcus sp., Staphylococcus sp.* at 500ppm and 1000ppm whereas no growth inhibition were observed with *Lactobacillus sp and Pseudomonas sp.* The methanolic extract of *Chrysanthemum leucanthemum* inhibited the growth of *Escherichia coli, Yersinia sp*. at 500ppm and 1000ppm, whereas *Pseudomonas sp., Lactobacillus sp., Enterococcus sp., Bacillus sp.* and *Klebsiella sp.* exhibited resistance to the extracts. The flower extracts of *Rosa centrifolia* was found to be effective against all the tested organisms with inhibition zone ranging from 0.4 to 0.6cm. The extract had a maximum inhibitory effect against *Lactobacillus* sp. (0.6cm) *and Pseudomonas* sp. (0.6cm). Moderate inhibitory activity against *Yersinia sp*. (0.55cm). A zone of 0.5cm was recorded against *Bacillus* sp., *Escherichia coli,*

Enterococcus sp., Klebsiella pneumoniae, whereas minimum activity was noted against *Staphylococcus sp*. (0.4cm). *Escherichia coli, Yersinia* sp., *Pseudomonas* sp., *Lactobacillus* sp., *Enterococcus* sp., *Bacillus* sp. and *Klebsiella* sp. did not show any inhibitory effect at 500ppm and 1000ppm of *Jasminum angustifolium* methanol extract. The blue colored methanolic extract of *Torenia fournieri* exhibited strong inhibitory action to *Lactobacillus sp* (0.4cm), *Pseudomonas sp* (0.4cm), *Yersinia sp* (0.3cm). A zone of 0.2cm was recorded against *Bacillus* sp.and *Enterococcus* sp. An intermediate effect was recorded against *Klebsiella* (0.15cm). The Gram negative rod, *Escherichia coli* and Gram positive *coccus, Staphylococcus* sp. showed a mild effect with a zone of 0.1cm.

Fig1. HPTLC analysis of methanolic extract of a) *Nerium indicum* b) *Tagetus erecta* c) *Rosa centrifolia* d) *Torenia fournieri*



a) **b)**

c) **d)**

Separation of active compounds by HPTLC:

Separation of active compounds from methanol extract of the selected flower were detected by Thin layer chromatographic plates using various solvent systems and analyzed in HPTLC **(Fig 1.)**. *Nerium indicum* methanol extract showed four compounds having an R_f value of 0.01, 0.13, 0.63 and 0.81 and λ max at 331, 327, 344 and 328. The compound 4 appeared to be the major compound with 42.87% area. *Tagetus erecta* methanol extract showed five compounds having an R_f value of 0.04, 0.11, 0.68, 0.82, 0.05 and λ max at 276, 277, 359, 359 and 277. The compound 5 was the major compound with 36.55% area. *Rosa centrifolia* methanol extract showed three

compounds having an R_f value of 0.07, 0.31, 0.47 and λ max at 548, 546 and 532. The compound 3 was observed to have an area of 49.65%. The methanolic extract of *Torenia fournieri* showed three compounds having an R_f value of 0.19, 0.23, 0.44 and λ max at 329,336 and 345 and compound 3 was observed as the major compound with an area of 36.87%.

DISCUSSION

The present investigation brings out adequate data on the antibacterial potential of methanol extract of various flower extracts. The study of compounds with antibacterial activity have targeted plants with a history of ethno bacterial uses [6-8]**,** while Herrera *et al*., [9] reported that only a few studies have targeted on randomly collected plants with localized distribution patterns. Findings of Araujo *et al.,* [10] demonstrated that antimicrobial properties of substances on desirable tools in the control of undesirable micro-organisms especially in the treatment of infection and in food spoilage. From the research of De Boer *et al.,* [11] it was noted that successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional or practitioners make use of water primarily as a solvent. The result from the present study revealed that methanol extracts of the selected plant was much better and powerful mainly due to the better solubility of the active compounds in organic solvents. This findings leads to the support of De Boer *et al***.,** [11] who demonstrated the better solubility of the active compounds in organic solvent. Furthermore Lin *et al.,* [12] has reported the effect of growth media on antibacterial activity. The present study was designed to obtain preliminary information on the anti microbial effect of the selected flower on certain pathogenic microorganisms. The well diffusion method was preferred to be used in this study to determine the antibacterial activity since it was found to be better than disc diffusion method; which also indicated that the sample volume taken to diffuse can be determined easily in contrast to the disc diffusion method, as it depends on absorbent paper. In this present study, the methanol flower extracts displayed a variable degree of antimicrobial activity against different test bacterial strains; whereas *Lactobacillus sp.* was found to be more sensitive strain to the entire tested flower extracts. The sensitivity pattern of *Yersinia sp.* ranged from a mild to moderate level for the methanolic extracts of *Torenia fournieri, Rosa centrifolia, Nerium indicum, Tagetus erecta* whereas entire resistant nature was noted for *Chrysanthemum leucanthemum, Jasminum angustifolium* extracts. Ayfer ates *et al.,* [13] reported that the acetone extracts of *Cinnamomum cassia* bark extracts showed no antibacterial against activity *Y. enterocolitica* whereas a zone of 15.5mm was obtained with respect to *T. bellerica* dry fruit crude extract. Astal *et al.,* [14] observed a zone of 10mm at a concentration of 20mg/ml of thyme extracts against *Pseudomonas aeroginosa* whereas in the present work, a maximum of 6mm inhibitory zone was recorded for *Rosa centrifolia* against *Pseudomonas sp.* An increased sensitivity of *Bacillus* sp. (17mm) was observed with respect to whole plant of *Achyranthes aspera* [15]**.** In the present study *Bacillus* sp. showed a 5mm zone in *Rosa centrifolia* extract at 1000ppm concentration. Jigna Parekh *et al.,* [16] recorded a zone of 10mm in the ethanolic extracts of *Lacenaea procumbens* against E.coli*;* whereas the results in the present study reveal that *E.coli* was sensitivity towards *Torenia fournieri, Rosa centrifolia, Tagetus erecta* flower extracts at 1000ppm concentration; and a maximum of 5mm zone of inhibition was found in *Rosa centrifolia* extract. The resistant nature of *Klebsiella* sp. was seen in *Nerium indicum, Chrysanthemum leucanthemum* and *Tagetus erecta* and a maximum of 5mm inhibitory zone was found in *Rosa centrifolia* extract. This finding supports the work of Pepeljnak *et al.*, [17] in which a higher inhibitory activity of 35mm zone for *Anisomelous mrlabariea* against *Klebsiella* sp. was found to

be resistant to *Nerium indicum, Chrysanthemum leucanthemum* and *Jasminum angustifolium* extracts. The inhibitory activity of *Enterobacter sp*. ranged from 2-5mm for a wide range of bacterial strains included in the testing. The activity against *Enterobacter* sp. for *Nerium indicum, Chrysanthemum leucanthemum* and *Jasminum angustifolium* extracts showed a nil spectrum. HPTLC analysis of the samples revealed a wide-variability in the methanolic extract. Out of the four extracts *Tagetus erecta* methanol extract showed five compounds.

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REFERENCES

[1] R Nair; T Kalariya; Sumitra Chanda. *Turk J Biol.*, **2005,** 29, 41-47.
[2] Parekh; Darshana Jadeja; Sumitra Chanda. *Turk J Biol,* **2005**, 29, 203-210.
[3] T Essawi; M Srour. *J Ethnopharmacol.*, **2000**, 70, 343-349.
[4] A V Krisharaj; T V N Rao; Sundararaju. *Int. J. Appl. Sci. Engg.,* **2005**, 2, 125-134.
[5] J N Eloff. *Plant Med.*, **1998**, 64, 711-713.
[6] E M Jovel; J Cabanillas; G H N Tower. *J. Ethanopharmacol.*, **1996,** 53, 149-152.
[7] R S L Taylor; F Edel; N P Manandhar; G H N Towers. *J. Ethanopharmacol.*, **1996**, 50, 197-102.
[8] J B Sindabiwe; M Calomme; P Cos; J Totte; L Pieters; A Vlientinck; D Vanden Berghe. *J. Ethanopharmacol.*, **1999**, 65, 71-74.
[9] K A Herrera; C F Carson; Riley; D A Pereztinherrara. *Phytother Res.*, **1996**, 10, 364-366.
[10] C M J Araujo; M F Sousa; Ferreria; C Leao. *J. Food Protect.*, **2003**, 66(4), 625-632.
[11] H J De Boer; A Kool; W R Mizirary; I Hedberg; J J Levenfors. *J. Ethanopharmacol.,* **2005**, 96, 461-469.
[12] J Lin, A R Opuku, M Geheeb-keller, A D Hutchings, S E Terblanche, A K Jager, M A Rassadah. *J. Ethanopharmacol*., **1998,** 68, 267-274.
[13] D Ayfer Ates; Ozlem Turgay Erdogrul. *Turk J Biol,* **2003**, 27, 157-162.
[14] Z Y E L Astal; A Aera; A A M Kerrit. *Pak. J. Med Sci.*, **2005**, 21(2), 181- 193.
[15] M R Rao; I B Reddy; T Ramana. *Indian Journal of Microbiology*, **2006,** 46, 259-261.
[16] Jigna Parekh; Sumitra Chanda. *African Journal of Biomedical Research,* **2006**, 9, 89-93.
[17] S Pepeljnjak; Z Kalodera; M Zovko. *Acta Pharm.*, **2005**, 55, 431-435.