WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 3, 729-736. Review Article ISSN 2277– 7105

EVALUATION OF ANTI-MICROBIAL PROPERTIES OF EXTRACTS OF *TANACETUM CINERARIIFOLIUM* (L.)

Menga Prasanna Sheela[1]*, Boga Ramya Sree[2] and Chelimela Narsaiah[2]

[1]Department of Biotechnology, University College, Telangana University, Nizamabad, Telangana, India-503322.

[2]Department of Pharmaceutical Chemistry, University College, Telangana University, Nizamabad, Telangana, India-503322.

Article Received on
10 Jan. 2019,

Revised on 31 Jan. 2019,
Accepted on 20 Feb. 2019

DOI: 10.20959/wjpr20193-14422

***Corresponding Author**
Menga Prasanna Sheela
Department of Biotechnology, University College, Telangana University, Nizamabad, Telangana, India-503322.

ABSTRACT

Since many years, plants were known to possess pharmacologically active compounds, can able to treat numerous diseases and disorders. In this paper, we report a study based on antimicrobial properties of plant *Tanacetum cinerariifolium* (L.). The leaves of plant were collected, shade dried and extracted with various solvents using Soxhlet extraction and hydrodistillation procedures. The crude extracts were evaluated for antibacterial activities against both Gram-positive and Gram-negative species; antifungal activity against *Candida* species. The extracts shown promising results against the tested microorganisms, further exploration need to be carried out to identify which chemical constituent is responsible for activity.

KEYWORDS: *Tanacetum cinerariifolium*, antimicrobial, antifungal and antibacterial.

1. INTRODUCTION

The genus *Tanacetum L.*, an important taxon of the Asteraceae family and found abundantly in Europe & western Asia and comprises of around 200 species. Numerous species of this genus have traditionally been used as a spicy additive for food, in cosmetics and as herbal medicines due to their biologically active compounds (Rohloff, 2004). Especially, *Tanacetum cinerariifolium* (L.) is a known remedy for the treatment of several diseases, which includes fever, arthritis, stomach-ache, vertigo, migraine, toothache, menstrual disorders, insect bites and psoriasis (Ernst, 2000). According to recent studies, essential oils and extracts of *Tanacetum* genus exhibit anti-inflammatory (Sur, 2009), anticancer (Zhang, 2005),

antibacterial (Holopainen, 1989), antiviral, antifungal (Holopainen, 1989), anthelmintic (Tiuman, 2005), insecticidal and antiprotozoal effects. Many studies have been reported on the essential oil composition of various Tanacetum species contains camphor, α-thujone, 1,8-cineole, carvone, thymol, trans-sabinyl acetate, caryophyllene oxide, borneol, (E)-myroxide, bornyl acetate, sabinene, isopulegone and artemisia ketone were identified as the major constituents. This genus is also found to contain sesquiterpene lactones, a large group of molecules with several biological activities.

T. cinerariifolium is an endemism growing in grazing lands of Sardinian and Corsican massifs, above 1300 m of altitude. Endemic taxa are very interesting because the geographic isolation has been caused a genetic and metabolic differentiation in these species, as shown by the high number of scientific researches that have been published until now [(Fattorusso, 2004) (Petitto, 2009) (Ramunno, 2005)]. Within a project aiming to find new agents with antibacterial activity the extract obtained by Soxhlet extraction (SE) with different solvents and volatile oils by hydrodistillation.

Volatile oils represent a small fraction of a plant's composition but confer them important characteristics to be used in the pharmaceutical, food and fragrance industries (Singh G, 2002). They have a complex composition, containing from a few dozen to several hundred constituents, especially hydrocarbons and oxygenated monoterpenes and sesquiterpenes (Pyun MS, 2006). It is important that the natural proportion of these components is maintained during extraction process (Dorman HJ, 2000). Hydrodistillation has traditionally been applied for essential oils recovery from plant materials. One of the disadvantages of this method is that essential oils undergo chemical alteration and the heat-sensitive compounds can easily be destroyed (Lucchesi ME, 2004). The extraction of volatile oils using solvents at high pressure, or supercritical fluids, has received much attention in the past several years, especially in food, pharmaceutical and cosmetic industries, because it presents an alternative to conventional processes such as organic solvent extraction and hydrodistillation (Bousbia N, 2009).

Thus, the aim of the present work was to compare the chemical composition of the extract obtained by SE and the essential oil obtained by hydrodistillation. Both the extract and essential oil were evaluated for their antimicrobial activity against different strains of bacteria and fungi. No studies have been found in the literature concerning the composition of the oil obtained from *T. cinerariifolium.*

2. MATERIALS AND METHODS

2.1. Preparation of extracts and dry residues

Extracts were prepared by adding 50 ml of boiling water to 5.0 g air-dried, coarsely powdered crude drug and heated in the Soxhlet apparatus for 5 hours with continuous stirring. Then the extract was covered with a watch glass, allowed to cool for 15 min, centrifuged and pressed through a sterile cellulose nitrate filter with a pore size of 0.2 pm. Extracts were tested immediately after preparation. The dry residue of an aqueous extract was determined by placing 5 ml of the sterile extract in a weighing bottle previously dried to constant weight at 105°C. Water was removed on a water bath and the residue dried under the same conditions. The extraction process was repeated with the solvents like Petroleum ether, Methanol & Ethyl acetate and obtained the powdered extract.

2.2. Test organisms

Mainly Gram-positive cocci and Bacillus subtilis as an aerobic spore forming bacterium were employed since these microorganisms are often involved in provoking infections. The following strains of bacteria were used as test organisms: *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 6057) and *Bacillus subtilis* (ATCC 9372) as Gram positive and *Escherichia coli* (ATCC 11,229) as an example of a Gram-negative bacterium. Two fungal species were used for testing *viz. Candida albicans* (ATCC 10231) *C. tropicalis* (ATCC 13803).

The bacteria were incubated on a nutrient agar slant (stationary culture) for 48 h at 37% followed by insulation in YET-Glucose Broth. This consisted of 0.5% yeast extract, 1% tryptone, 1% glucose and 1% sodium chloride (pH 7.0). The turbidity was corrected by adding isotonic sodium chloride solution until a McFarland turbidity standard of 0.5 (lo8 colony-foxing units, CFU/ml) was achieved in an Turbidometer.

2.3. Antimicrobial testing

In a preliminary microbiological study, the extracts were tested by means of the ‘stroke method’. Sterile nutrient agar with plant extract in a ratio of 8:2 was prepared. This mixture was poured into a Petri dish under aseptic conditions and allowed to cool for 30 min at room temperature. For inoculation of the microorganisms, small strokes of the cultures were applied to the surface of the agar medium. The plates were incubated at 37°C for 24 h after which the results were rated by the unaided eye as: strong inhibition (no growth), partial inhibition (less growth than normal) and no inhibition (full growth). Once an extract showed

a definite inhibitory effect, further testing was carried out by means of the 'hole-plate diffusion method' (HPD). In accordance with preliminary investigations, this method turned out to be the most suitable one.

Antibacterial activity

Muller Hinton Agar was prepared according to the manufacturer's instructions. The medium was sterilized by autoclaving at 121°C for 15 minutes at 15 psi pressure and was used to determine the antibacterial activity of the extracts. Sterile molten cool (45°C) agar was poured aseptically into sterile Petri plates (15 ml each) and the plates were allowed to solidify at room temperature in a sterile condition. After solidification and drying, the plates were seeded with appropriate microorganisms by spreading evenly on to the surface of the medium with a sterile spreader and wells (8 mm diameter) were cut out from the agar plates using a sterile stainless-steel bore and filled with 0.1ml of the extract solutions in respective wells. Tetracycline and double distilled water were used as positive and negative control respectively. Then the plates were incubated at 37°C for 24 hrs in the next day the zones of inhibition were measured with a measuring scale. This experiment was carried out in triplicate for their confirmation. The results were read by the presence or absence of zone of inhibition.

Antifungal activity

Sabouraud Dextrose Agar medium was prepared by dissolving required quantities of Peptone and Dextrose. The volume was made up and pH was adjusted to 5.6 ± 0.2 using 0.1N sodium hydroxide. Agar was added and dissolved by heating. The prepared media was measured 20 ml and transferred to each boiling tube and sterilized by autoclaving at 15lb/in² for 20 about minutes. The testing samples were prepared in different concentrations of 10, 20, 30, up to 100 µg/ml in methanol. The prepared concentrations of sample were added to the medium and poured into Petri plates and allowed to solidify at room temperature. The plates were divided into four equal parts on outer surface with glass marker and code each part with the specific organism. 24 hr cultures of fungi were streaked at the specified area on the Petri plate. Growth/inhibition was measured after 72 hrs of incubation 37 ± 1^{0}C.

Sterile nutrient agar was poured into Petri dishes. The agar was left to set, and 0.8 ml of the appropriate bacterial suspension was distributed on the agar. A 10 mm core of agar was removed from the seeded agar and the hole was closed against the dish bottom with pure agar. Five wells were aseptically filled up by means of a pipettor with 220 µl of each plant

extract, while an equal amount of tetracycline HCl in physiological tris buffer (pH 7.4) was placed in the sixth hole as a reference standard.

The solutions of the extracts and the standard were arranged in form of a Latin square, alternated around the dish. After incubation for 24 h at 37°C the diameter of the inhibition zones was measured in mm. Two sets of control were used. One control was the organism control and consisted of a seeded Petri dish with no plant material or standard. In the second control, plant material and standard were introduced in the holes of unseeded Petri dishes to check for sterility.

The degree of bacterial inhibition of the extracts was determined using the 'microdilution broth method' (MDB) to establish the minimal inhibitory concentration (MIC). The plant extracts were diluted with Mueller-Hinton broth to obtain concentrations of 90, 80, 70, 60,40, 30, 20, 15, 10, 7.5, 2.5 and 1%. 200 µ1of each dilution and 2 x 200 µl of Mueller-Hinton broth without plant extracts (positive and negative controls) were brought onto a microtiter plate to obtain 10 serial dilution wells and 2 control wells in one column. For each tested organism 2 rows were used in a parallel experiment. Tetracycline HCl, as reference for determination of MIC, was used in a concentration of 8-10 µg/ml.

3. RESULTS AND DISCUSSION

Determination of the inhibition zones by means of the HPD method and of the MIC with the aid of the MDB method showed that about 60% of the plant extracts tested exhibited a pronounced antibacterial effect against one or more of the 4 test bacteria. The antimicrobial effect, expressed as MIC, was found in concentrations of 6-19 mm against Gram-positive and of 6-15 mm against Gram-negative bacteria (Table 1), whereas antifungal activity, the extracts were shown 5.9-15.1 µl against *C. albicans* and 9.5-16.3 against *C. tropicalis*.

The diameters of the inhibition zones obtained using the HPD method did not in all cases confirm the results of the MDB method. This difference may be due to the diffusion behavior of the extracts. Transport of a drug through intact biomembranes depends to a great extent on the lipophilic properties of a substance. For a full interpretation of the present results further investigations are necessary to elucidate the influence of the dissociation constant, the solubility, molecular size and other factors such as the blood flow of the tissue, pH at the site of action and the local distribution of active substances and extracts.

Table 1: Zone of Inhibition of the plant extracts in antibacterial activity.

Antibacterial activity- Zone of Inhibition				
	S. aureus	***B. Subtilis***	***E. Coli***	***E. faecalis***
Standard	**Ampicillin**	**Norfloxacin**	**Ceftriaxone**	**Ciprofloxacin**
10	8 mm	10 mm	19 mm	09 mm
25	10 mm	15 mm	22 mm	14 mm
50	14 mm	13 mm	27 mm	20 mm
Water				
10	06 mm	0 mm	06 mm	08 mm
25	09 mm	02 mm	09 mm	10 mm
50	12mm	06 mm	12mm	17 mm
Pet. Eth.				
10	08 mm	10mm	12 mm	06 mm
25	15 mm	15mm	12 mm	09 mm
50	18 mm	13mm	13 mm	12 mm
Meth.				
10	11 mm	09 mm	10 mm	05 mm
25	15 mm	13 mm	14 mm	09 mm
50	19 mm	14 mm	15 mm	13 mm
Et. Acet.				
10	08 mm	02 mm	06 mm	08 mm
25	09 mm	05 mm	09 mm	10 mm
50	11 mm	06 mm	13 mm	11 mm

Table 2: MIC values of the plant extracts in antifungal activity.

Antifungal activity- MIC (µL)		
Sample	***C. albicans***	***C. tropicalis***
Fluconazole	5.5-6.0	9.4-11.2
Water extract	10.0-11.6	15.5-16.3
Pet. Eth. Extract	14.0-15.1	13.3-14.6
Meth. Extract	5.9-6.8	9.5-11.2
Et. Acet extract	8.7-10.2	12.6-13.1

The results are given in Table 1 & Table 2 represents the summary of antimicrobial activities of the various extracts with respect to each of the test organisms. The responsible antibacterial agent might be any of the following: camphor, α-thujone, 1,8-cineole, carvone, thymol, trans-sabinyl acetate, caryophyllene oxide, borneol, (E)-myroxide, bornyl acetate, sabinene, isopulegone and artemisia ketone. A search of the literature indicated that these compounds have possessed the antibacterial property. These constituents belongs to triterpene saponins and the polyacetylene falcarinol, Enpentain and the naphthoquinone plumbagin. The antibacterial effects of the extracts may also because of polyphenolic substances, tannins, catechins and polyphenolic acids. It is known that polyphenols can form heavy soluble complexes with proteins. Polyphenols may bind to bacterial adhesins and by

doing so they disturb the availability of receptors on the cell surface. The literature demonstrates that antibacterial activity can also be due to tannins.

Another aspect of this plant is, it contains considerable amounts of essential oils. In adequate concentrations, most of the essential oils are able to damage microorganisms. The extracts exhibited a marked activity or a broad spectrum of activity, especially toward the gram-negative *E. coli*. These results suggest the presence of either good antibacterial potency or of the high concentration of an active principle in the extract.

4. CONCLUSION

Some vegetal extracts tested in this work showed a considerable antibacterial and antifungal activity. It is an interesting fact that some extracts are active on some plant pathogens. From these findings, the final conclusion is that the *Tanacetum cinerariifolium* extracts are useful for systemic use on animals and human beings, but they could be considered interesting for use as antimicrobial drugs on superficial microbial infections, such as staphylococcal pyodermitis or mucosal candidiasis, after adequate *in vivo* animal and clinical tests. Another important consideration is that the substances tested in this work were crude vegetal extracts that contain a mixture of several compounds. It would be interesting to prepare pure single compounds of these plants, with the aim to find out some molecules with low toxicity and an improved antimicrobial activity.

Conflict of Interest

The authors declare no conflict of interest in publication of this paper.

ACKNOWLEDGEMENT

Authors wish to thank the Registrar, Telanagana University, Nizamabad for the support during the research work.

REFERENCES

1. Bousbia N, V. M. Comparison of two isolation methods for essential oil from rosemary leaves: Hydrodistillation and microwave hydrodiffusion and gravity. *Food Chemistry*, 2009; 355-362.
2. Dorman HJ, D. S. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of applied microbiology*, 2000; 308-316.

3. Ernst, E. P. The efficacy and safety of feverfew (Tanacetum parthenium L.): an update of a systematic review. *Public Health Nutr*, 2000; 509–514.
4. Fattorusso, E. S.-S. Polyoxygenated eudesmanes and trans-chrysanthemanes from the aerial parts. *J. Nat. Prod.*, 2004; 37-41.
5. Holopainen, M. K. Antimicrobial activity ofdifferent chemotypes of Finnish Tansy. *Planta Med.*, 1989; 103-108.
6. Lucchesi ME, C. F. Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro-distillation. *ournal of Chromatography A*, 2004; 323-327.
7. Petitto, V. S. Iridoids from Euphrasia genargentea, a rare Sardinian endemism. . *Nat. Prod. Res.*, 2009; 431-435.
8. Pyun MS, S. S. Antifungal effects of the volatile oils from Allium plants against Trichophyton species and synergism of the oils with ketoconazole. *Phytomedicine*, 2006; 394-400.
9. Ramunno, A. S. Taxonomical merkers in two endemic plants of Sardinia: Verbascum conocarpum and Scrophularia trifoliata. *Nat. Prod. Res.*, 2005; 142-149.
10. Rohloff, J. M. Chemotypical variation of tansy (Tanacetum vulgare L.) from 40 different locations in Norway. *J. Agric. Food Chem*, 2004; 1742-1748.
11. Singh G, K. I. Studies on essential oils: part 10; antibacterial activity of volatile oils of some spices. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives.*, 2002; 680-682.
12. Sur, R. M. Anti-inflammatory activity of parthenolide-depleted feverfew (Tanacetum parthenium). *Inflammopharmacology*, 2009; 42-49.
13. Tiuman, T. U.-N.-D. Antileishmanial activity of parthenolide, a sesquiterpene lactone isolated from Tanacetum parthenium. *Antimicrob. Agents Chemother*, 2005; 176-182.
14. Zhang, S. W. Anti-cancer potential of sesquiterpene lactones: bioactivity and molecular mechanisms. *Curr. Med. Chem. Anticancer Agents*, 2005; 239-249.