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Original Article

Development and Evaluation of Antimicrobial Herbal Gel Formulation

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ABSTRACT

Objective: In the developing countries there has been a gradual revival of interest in the use of medicinal plants in the recent years, credited to the safety, paucity and minimal side effects of the herbal drugs. **Experimental approach**: The present study is an attempt to formulate two drugs *Tridax Procumbens* and *Galinsoga Parviflora* into ethosomal gels individually and in combination of two herbs and evaluate the anti microbial activity in comparison with the standard drug. Ethosomes of *Tridax Procumbens* and *Galinsoga Parviflora* were produced using phospholipid ,cholesterol, ethanol, and propylene glycol which are further incorporated in to gel made with carbopol 934. The formulation were evaluated for anti-microbial activity. **Findings;** The highest activity was shown on *Pseudomonas aeroginosa* with *zone of inhibition* 31.1(mm). A potentiation in the anti-microbial activity was observed in combination and significant activity P<0.01was established when compared with standard drugs. **Discussion**: The herbal drugs though numerous in nature, with anti-microbial activity were insignificantly established as potential drugs for use, when compared to standard synthetic drugs. Ethosomal vesicles are used for drug delivery into deep skin layers and the systemic circulation. These are the advanced forms of liposomes with high ethanol content, in which hydrophilic and hydrophobic drugs can be incorporated, thus enhance the accommodation of drugs. The ethosomal drug is administered in semisolid form hence producing high patient compliance. **Conclusion:** Ethosomal gel formulations for herbal drugs with varied phytoconstituents, with varying solubility, are suitable transdermal drug delivery systems and using two herbal extracts has synergized the antimicrobial potential.

Keywords: *Tridax Procumbens, Galinsoga Parviflora,*Ethosome gel, Anti-microbial activity.

1. INTRODUCTION

Traditional medicine, since ages has been an important source of potentially useful new compounds to develop chemotherapeutic agents and nature is contributing to an impressive number from which number of modern drugs have been isolated.[1]

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The emphasis on development of biologically active new molecules has been gradually replaced by the use of total herbs as medicine and food supplements. High treatment costs and side effects along with drug resistance are major problems associated with synthetic drugs and they can be overcome by the use of traditional medicine.[2]

In the recent times there is an immense increase in the awareness, recognition and development of the medicinal and economic benefits of traditional medicinal plants in the developing countries. Extensive screening of medicinal plants for antimicrobial activity has been carried out but with less attention on the development and improvement of herbal formulations with enhanced pharmacological activities to compete with the synthetic drug formulations with higher potential.[3] Medicinal plants with potential antimicrobial activities have to be explored because of their immense need, as there is an increase in the failure of synthetic drugs, side effects and development of antibiotic resistance by pathogenic microorganisms.[4] Folk-lore medicine has revealed the utility of numerous plants for their antimicrobial potential and demonstrated the method of extraction and the administration of extractives internally and externally. There still remains a lacuna and shows a difference in the active potentials of the plant extracts invitro and invivo.

The antimicrobial agents used to treat the superficial infections and infected wound in the wound care management to maintain and produce an antisepsis have to be formulated as topical applications and the limiting factors i.e, epidermal barrier, drug availabilities and retention over the required site remains valid for formulating the transdermal drug delivery systems.

Even after exhibiting the promising therapeutic effects, most of the phytoconstituents fail to achieve bioavailability for reasons like large molecular sizes and low lipid solubility and there by poor absorption and reduced bioavailability.[5]

Incorporation of the plant actives or extracts in to vesicular carriers greatly improves their absorption and consequently bioavailability.

Ethosomal vesicles are used for drug delivery into deep skin layers and the systemic circulation. [6] These are the advanced forms of liposomes with high ethanol content, in which hydrophilic and hydrophobic drugs can be incorporated, thus enhance the accommodation of drugs. The ethosomal drug is administered in semisolid form hence producing high patient compliance.[7]

Weeds are not really 'unwanted' especially in terms of traditional herbal medicines. some of these "naturally growing plants" which are generally known as group of very aggressive, noxious, competitive and trouble some plants are used by ethno-medical practitioners for their possession of significant medicinal properties. Two such plants are *Tridax Procumbens* and *Galinsoga parviflora.*

Tridax P. commonly known as 'coat buttons' because of the appearance of its flowers belonging to the family asteraceae.[1] The common weed showing distribution in most parts of the world is employed as indigenous medicine for variety of ailments , extensively used in Ayurveda as 'Bhringraj', a medicine for liver disorders. It has been found to posses significant medicinal properties against blood pressure, malaria, dysentery, diarrhoea, wound healing, prevents hair fall check haemorrhage show pharmacological activities like immunomodulatory, Anti inflammatory, antiseptic, anti hepatotoxic, antioxidant and analgesic.[8] The chemical constituents reported were alkaloids, flavanoids, carotenoids, sterols, glycosides, tannins, phenolic acids.

Galinsoga genus belongs to family Asteraceae, is represented with 13 species. *G. parviflora* commonly called as Gallent Soldier shows distribution in most of the India and many parts of the world.[9] Leaf paste as external application, is useful in treating nettle sting and other inflammations. This finds application in the treatment of cuts and wounds, snake and scorpion bites, against dysentery and blood stools. The chief chemical constituents present are alkaloids, flavanoids, terpenoids, phenols, saponins.

The safety of the herbal drugs is evident as they are used as food for humans and fodder for cattle and hence non-toxic. When related to the antimicrobial therapy, it is important to know the effect of combination therapy as it has numerous benefits including treatment of infections caused by specific causative organisms, improving the antimicrobial effects, improving the spectrum of activity and reducing the resistance for the drugs by the causative organisms. The potentiating of the activity must be due to the different phytoconstituents present exhibiting multifaceted action on different components of the system which consolidates to enhancing of the activity.

2. MATERIALS AND METHOD

Collection of plant material and preparation of Extract:

The plants were collected; shade dried, coarsely powdered and stored in air tight containers. The Botanical authentication of both the herbs were done at the Department of Botany, Yogi Vemana university , kadapa , Andhra Pradesh.

Monographic analysis of Herbs:

Herbs were evaluated for the extractives values, ash values, and loss on drying according to standard specifications in Herbal Pharmacopoeia of India.

Extraction:

Shade dried aerials parts of *T.Procumbens* and *G.Paviflora* were subjected to extraction with ethanol by maceration for 48 hrs followed by filtration and evaporation using rota evaporator under reduced pressure. The herbal extract concentrates were stored in desiccator until used.

Preliminary phytochemical screening:

The extracts obtained were evaluated for phytoconstituents using standard procedures.

Preparation of Ethosomes:

Accurately measured quantities of lipid and cholesterol were dispersed in water by stirring for 30 minutes on a magnetic

stirrer and heating at 40° C. Organic phase containing extract weighed measure of extract was added to ethanol and to that propylene glycol was added. Lipid solution was added drop by drop to the organic phase and kept stirring for 1 hr.

Preparation of Ethosomal Gel:

Gels were prepared by dispersing gelling agent to distilled water and allowing to swell overnight. The mixture was neutralised by dropwise addition of triethanolamine. Then glycerol was added to gel to balance viscosity.[10] Mixing is continued until a transparent gel appeared. Paraben was added as a preservative and stored at 4-8° C.[11]

Evaluation of prepared gels:

Physicochemical properties:

The colour, appearance and the feel on application of the formulated gels were noted. The properties such as consistency and texture were checked. The pH was measured using digital pH meter.[12]

Spreadability of gels was determined by measuring the spreading diameter of 1g of gel between two horizontal plates (20 cm x 20 cm) after 1 min with the standard weight of 125 gm on upper plate.

Viscosity was measured using Brookfield viscometer.

Acute skin irritation was performed following OECD guide lines.

Drug content of the formulated gels was estimated spectophotometrically. 100mg of the formulation was taken and dissolved in methanol and filtered. Absorbance was measured at 212 nm after suitably diluted.

Anti-microbial evaluation:

Determination of anti-microbial activity and anti-fungal activity:

Preparation of inoculums:

24 hrs old fresh cultures of fungi and bacteria were suspended in sterile water to obtain a uniform suspension.[13]

Inoculums of two Gram '+' ve , two Gram '-' ve, and one fungi were prepared.

Gram '+' ve organisms : *Staphylococcus aureus*, *Bacillus subtilis*.

Gram '-' ve organisms : *Escherichia coli*, *Pseudomonas aeruginosa*.

Fungi : *Candida albicans*.

Determination of zone of inhibition;

Anti-microbial and anti-fungal activities were determined by agar well diffusion method. In this method, suitable sterile nutrient media still in molten state at 40° C were inoculated with respective microorganisms, i.e, inoculum is added appropriately and poured into sterile petriplates following aseptic techniques.

Culture medium is allowed to solidify. Care was taken for the uniform thickness of the layer of medium in different plates. Wells were made in the solidified agar plates using sterile cork borer. The ethosomal gels of individual extracts and the formulated ethosomal gels with 2:1 ratio of *Tridax* and *Galinsoga* vesicles, these dissolved in to appropriate solvent, the DMSO and placed carefully into the pits in the plates that were previously inoculated with selected bacteria and fungi. The plates were kept for pre-diffusion for 30 min.[14, 15]

The suitable media for the growth of bacteria is nutrient agar and that for fungi is saborouds agar medium. After normalization to room temperature the plates of bacteria were incubated at 37° C for 24 hrs and at 25° C for 48 hrs in case of fungi. After incubation period, the zones of inhibition were measured.

The zone of inhibition of the formulated ethosomal gels was compared with that produced by the standards.

Standard drug for bacterial activity- Ampicillin

Standard drug for fungal activity- Fluconazole

Statistical analysis:

The results of the studies were expressed as mean ± S.D, n=3. Probability values, $p<0.01$ were considered significant when compared to standard.

3. RESULTS AND DISCUSSION

The monographic analysis of herbs and preliminary phytochemical screeninig of ethanolic extract of the herbs were conducted and results were depicted in the table 1 and 2.

Table 1: Monographic analysis of selected herbs

S.no	Parameter	% w/w (Mean)	
		T.Procumbens	*G.Parviflora*
1	Loss on drying	4.1%	5.2%
2	Water soluble ash	4.1%	4.9%
3	Acid soluble ash	3.9%	3.4%.
4	Alcohol soluble extractives	5.8%	5.1%
5	Total ash value	18%	21%
6	Sulfated ash value	21.3%	22.3%

Preliminary phytochemical screening of *T.Procumbens* and *G.Parviflora*:

Table 2: Phytochemical evaluation

Phyto chemicals	T.Procumbens	G.Parviflora
Alkaloids	-	+
Phenols	+	+
Flavanoids	+	+
Sterols	+	-
Terpenoids	+	+
Tannins	+	+
Saponins	+	+

'+' Present ; '-' Absent.

Ethosomal vesicles were developed to incorporate into the gel formulation. The ethosomes of two concentrations of *Tridax* and two concentrations of *Galinsoga* were developed and with these ethosomes, Herbal gels were formulated.

F_1 is the gel formulation with 1% of ethosomes of 300 mg concentration of *Tridax Procumbens*.

F_2 was the gel formulation with 1% of ethosomes of 300 mg concentration of *Galinsoga Parviflora.*

F_3 was the combined gel formulation with 1% of ethosomes of 100mg concentration of *Tridax Procumbens* and 200 mg of concentration of *Galinsoga Parviflora* in a ratio 1:2.

Table 3: Formulation of Ethosomes

S.no	Ingredients	% w/w
1	Carbopol 934	0.3
2	Propylene glycol	20
3	Glycerin	5
4	Proply paraben	0.02
5	Methyl paraben	0.18
6	Triethanolamine	Q.S to neutralise
7	Ethosomal vesicles	1

Combionation gel formulation was made using 100 mg concentration ethosomes of *Tridax* and 200 mg concentration ethosomes of *Galinsoga*.

Table 4: Formulation of Ethosomal Gels

Formulation	Drug content (mg)	Lecithin (mg)	Cholesterol (mg)	Ethanol (ml)	Propylene glycol (ml)
Tridax Ethosomes	300 100	300 300	30 30	20 20	3 3
Galinsoga Ethosomes	300 200	300 300	30 30	20 20	3 3

Acute toxicity studies were conducted for 14 days following OECD guidelines, observed for erythema and oedema.

Table 5 : Evaluation of physicochemical properties of Ethosomal formulations

Formulation	Colour	Appearence	Spreadability g.cm/se	P^H	Viscosity cps	Drug content (%)
Tridax gel	Faint green	Homogenous	38.54	6.5	3476	81.42
Galinsoga gel	Dark green	Homogenous	33.72	6.6	3392	79.87
Combinationgel	green	Homogenous	34.65	6.6	3459	82.31

Anti-microbial activity of the herbal gels was evaluated and compared with the marketed standard.

Table 6 : Evaluation of anti-microbial activity

Test organism	Zone of Inhibition (mm)				
	T.Procumbens (1 % w/w)	*G.parviflora* (1 % w/w)	Combination gel(1% w/w)	Ampicillin (100µg/ml)	Flucanazole (50µg/ml)
S.aureus	± 14.7	± 24.4	± 26.3	± 23.0	-
B.sbtilis	± 13.9	± 20.2	± 23.1	± 22.8	-
E.coli	± 23.7	± 20.1	± 25.7	± 20.1	-
P.aeroginosa	± 16.2	± 29.0	± 31.1	± 21.2	-
C.albicans	± 11.1	± 20.8	± 22.6	-	± 19.3

Note: The data are expressed in M**ean ± S.D** : n= 3, p<0.01, significant compared to standard drug.

Figures:

Figure (a): **Figure (b):**

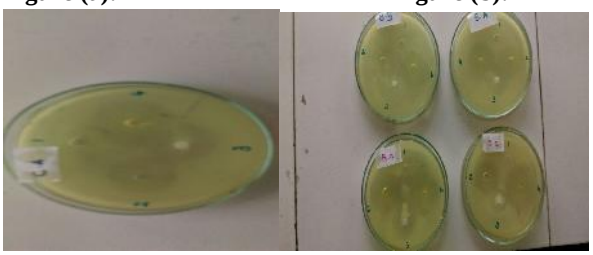

Fig 1: Zone of inhibition diameters Zone of inhibiton diameters of *B.subtilis, S.aureus, E.coli,* of *Candida albicans*. *P.aeroginosa*

Discussion:

Majority of the herbal products in Indian system of medicine are made using the crude extracts and directly incorporating them in to a form convenient for dispensing. In the attempts to improve their potential and tap them to the optimum extent, by improving the bioavailability and avoiding the limitations of drug entrapment and release, the novel drug delivery systems were developed. The one amongst that which acquired great popularity for its advantages is the ethosomal drug delivery system.

The herbal drugs though numerous in nature, with anti-microbial activity were insignificantly established as potential drugs for use, when compared to standard synthetic drugs. Exploring the ways to place these potential elements into an efficient vehicle which can transport them to the prime site is the need of the day, so that the actual potential of the active elements can be ultimately utilized.

The ethosomal gels formulated were found to exhibit good gel characteristics. % drug content present estimated by spectrophotometric method reveals that the entrapment efficiency of ethosomal vesicles formulated was good and drug release was significant. The antimicrobial activity of the ethanolic extract of *Tridax* further which was made into ethosomal gel was very less when compared with the standard drugs. *Galinsoga parviflora* gel showed moderate activity and that is enhanced when it is formulated in combination with *Tridax procumbens*.

F_3- combined formulation showed highest zone of inhibition for *Pseudomonas aeruginosa* and all other values have increased which evidently prove that both factors, the combinations of drugs and their form of drug delivery system have together worked out to potentiate the activity. The *Galinsoga* with proven antimicrobial, anti oxidant , and anti-inflammatory activities when taken in combination with *Tridax* with moderately potential antimicrobial activity but with significant immunomodulatory and wound healing activities has produced modest herbal formulation with enhanced anti-microbial activity to compete with the marketed synthetic antimicrobial drugs.

4. CONCLUSION

The present study demonstrated that the herbal ethosomal gel, formulated with combination of herbal extracts

possesses significant topical anti-microbial properties. The combination has a scope for the further study of anti-protozoal and antileprotic activities as the pharmacological activity screening on both the plants revealed such potentiality.

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6. REFERENCES

1. Jain A, Amita J. Tridax procumbens (L.): A weed with immense medical impotance: A review. International journal of pharma and bio sciences. 2012; 3(1): 544-552
2. Nitin G, Ankit R P. Design of Akkalkara (Spilanthes acemella) formulations for antimicrobial and topical anti-inflammatory activities. International journal of pharma and bio sciences. 2012; 3(4): 161-170
3. Sailaja PR, Bala TS, Sireesha K. Formulation and Evaluation of Gel containing Mangifera indica leaves extract for antimicrobial activity. International Journal of Pharmacognosy. 2018; 5(1): 61-68
4. Ateb D A, Erdourul T. Antimicrobial activities of various medicinal and commercial plant extracts. Turk J Biol. 2003; 27:157-162
5. Nimisha , Nimitha. Development and evaluation of herbal cosmoceutical for skin care. International Journal of Pharma and Bio Sciences. 2013; 4(2): 86-92
6. Nimmy J K, Krishna kumar, Dinesh K B, Smitha K N. Ethosomal gel: A review. European Journal of Pharmaceutical and medical research. 2017; 4(4): 301-305
7. Indira S, Reddymalla P, Srinivas P. Formulation and evaluation of Ethosomal topical gel of Etoricoxib. International Journal of Pharmaceutical Research scholars. 2015; 4:1-4
8. Anil S, Harish K S, Parvesh G. A review on Tridax procumbens. Imperial Journal of Interdisciplinary research. 2016; 2(8): 308-319
9. Patharaj J K. Phytochemical analysis of Gallant soldier (Galinsoga parviflora) cav. from Nilgiris of India. International journal of Research in Pharmacy and Pharmaceutical sciences. 2017; 2(4): 76-78
10. P Sailaja rao, Bala Tripura sundari, Sireesha K, Prakash V Diwan. Evaluation of Ethosomal gel formulation of mangifera indica on excision wounds in wistar rats. International journal of scientific research. 2017; 6(11): 292-295
11. Tarun P, Soniya, Roopesh et al. Ethosomes: A recent vesicle of transdermal drug delivery system. International Journal of Research and Development in Pharmacy and Life Sciences. 2013; 2(2): 285-292
12. Samar A, Sara Z, Mohammad Y. Ethnobotanical, phytochemical and pharmacological properties of Galinsoga parviflora (Asteraceae): A review. Tropical Journal of Pharmaceutical Research. 2017; 16(12): 3023-3033
13. Abhijeet P, Jui V J, Poishettiwar S A. Formulation and Evaluation of In-vitro antimicrobial activity of Gel containing essential oils and effect of polymer on their antimicrobial activity. International Journal of Pharmacy and Pharmaceutical sciences. 2011; 3(1): 234-237
14. Abu-Shanab B, Adwan g, Abu safiyan D et al. Antimicrobial activities of some plant extracts utilized in popular medicine in Palestine. Turk J Biol. 2004; 28: 99-102
15. Jadhav V D, Swati T, Akshada B, Chaudhari G N. Formulation and evaluation of Herbal gel containing leaf extract of Tridax procumbens. Journal of Pharmaceutical and Biosciences. 2015; 3: 65-72 .

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