



FORMULATION AND EVALUATION OF POLY HERBAL EXTRACT AGAINST DIABETIC FOOT INFECTION

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ABSTRACT

Natural remedies are more acceptable in the treatment of different types of diseases when compared to synthetic ones. Why because it was believed that herbal remedies are safer with fewer side effects. So the demand of herbal formulations has growing in the world market. The present study deals with the formulation and evaluation of Anti microbial activity of Poly herbal formulation containing ethanolic extract of *Gymnema sylvestre*, *Allium sativum*, *Psidium guava*, *Centella asiatica*, *Curcuma longa*, *Trigonella foenum*, *Acalypha indica*, *Momordica charantia*, *Zingiber officinale*, *Rosmarinus officinalis*. These plants have been reported in the literature having good anti diabetic, anti microbial, anti oxidant, anti inflammatory, wound healing activity, anti fungal, anti cancer, anti ulcer, analgesic and anti diuretic activity. So there is need to selection of such drugs and formulated as poly herbal formulation in the treatment of diabetic foot infection. The prepared poly herbal extract was tested for the anti microbial activity against micro organisms such as gram positive bacteria include *Staphylococcus aureus*, *Bacillus subtilis*, gram negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* respectively by using modified agar well diffusion method. The prepared poly herbal extract was effectively tested against microorganism which was well comparable with standard antibiotic. The ethanolic poly herbal extract showed the zone of inhibition indicating that the plants used in the formulation were fight against these microorganisms due to the presence of tannins and also it could be better alternative to the modern medicine. In majority of traditional systems of medicine, diabetes is better managed by the combination of herbs (Polyherbal) instead of single herb because of synergism and less side effects.

Keywords: Diabetic foot infection, poly herbal extract, anti microbial activity, modified agar well diffusion method.

1. INTRODUCTION:

Herbal drugs usage has recently been increased throughout the world for the maintenance and improvement of health and for the treatment of various disorders and diseases. Approximately 60% of the world population and 60–90% of the population of developing countries depends on traditional medicine for their primary health care. About one-third of all traditional medicines now in usage have been for the treatment of wounds and skin disorders, compared to only 1–3% of modern drugs. These show a clear indication for the important role of medicinal plants as therapeutic alternatives to conventional medicine^[1].

Foot infections are common in people suffering with diabetes mellitus. Most diabetic

foot infections occur in a foot ulcer, which plays a significant role in the worsening of the ulcers and can lead to the formation of gangrenous wounds^[2]. If remain untreated, infection can spread to underlying tissues, including bone. A diabetic foot infection is the main clinical consequence leading to lower extremity amputation, and about 60% of all amputations in developed countries^[3].

Investigations from the bacteriological studies says that the diabetic foot infections were commonly associated with polymicrobial etiology. Diabetic foot infections are often complex and polymicrobial in nature. On an average 2.4 organisms recovered per wound. Of the infected diabetic foot ulcers, 75% had multiple micro organisms^[4].

Diabetic foot ulcer is a result of repeated infections due to aerobes, anaerobes or fungi individually or in combination^[2]. Aerobes (Gram + negative) such as *Staphylococcus*

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aureus (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) are seen as pathogens in diabetic wounds [5]. Gram – negative aerobes such as the *Citrobacter sp.*, *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Acinetobacter* and *Serratia sp.* are often cultured from a diabetic wound [6]. Most Gram - positive anaerobes include the *Peptostreptococcus* spp., *Clostridium* spp., along with other *Propionibacterium* spp., [7]. Common Gram – negative anaerobes are *Bacteroides fragilis*, *Prevotella* species, *Fusobacterium* species and *Veillonella* species [4]. Most common clinically significant fungi cultured from DF ulcers is *Candida* spp. [6]. The *Candida* species isolated from diabetic foot ulcers include *C. tropicalis*, *C. albicans*, *C. guilliermondii* and *C. pseudotropicalis* [8]. Communities of microbial cells growing on a surface and embedded in a self-synthesized matrix composed of extracellular polymeric substances leads to biofilm formation [9]. Biofilms are found to be present in two-thirds of chronic wounds which are resistant to antibiotics [10]. Reports says that *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were the most common single agents causes diabetic foot infection [11,12,13,14].

Among persons having diabetes mellitus, the prevalence of foot ulcers is 4–10%. Despite of all efforts in treatment of diabetic foot ulcers, the rate of amputation of lower extremities is still high and there is a need for effective and safe treatments to prevent amputations, to improve quality of life, to control infection and to reduce treatment cost [15].

The plants were used in the preparation of poly herbal extract *Gymnema sylvestre*, *Allium sativum*, *Psidium guava*, *Acalypha indica*, *Momordica charantia*, *Trigonella foenum-graecum*, *Zingiber officinale* *Curcuma longa*, *Rosemarinus officinalis*.

Acalypha indica is reported to possess rat cutaneous wound healing property in experimental models [16]. *Allium sativum* is reported to possess anti microbial property against micro organisms causing diabetic foot infection [17,18]. Plants such as *Gymnema sylvestre*, *Allium sativum*, *Momordica charantia*, and *Trigonella foenum-graecum* are

reported to possess curative effect against diabetes and its complication [19]. *Psidium guajava* is reported to possess anti microbial property against organism responsible for causing diabetic foot infection [20,21] and excellent anti hyperglycaemic effect [22]. *Curcuma longa* is reported to have wound healing activity by increasing the levels of beta transforming growth factor plus an increase in the activity of the enzyme nitric oxide synthase [23]. *Rosmarinus officinalis* L was reported to possess antidiabetic and antioxidant and activities [24].

2. MATERIALS AND METHODS:

Collection and authentication of plant material

The leaves of *Gymnema sylvestre* R.Br. (Asclepidaceae) (PARC/2013/2091), *Psidium guajava* (Myrtaceae) (PARC/2013/2093), bulbs of *Allium sativum* L (Liliaceae) (PARC/2013/2086), rhizomes of *Curcuma longa* (Zingiberaceae) (PARC/2013/2089), *Zingiber officinale* (Euphorbiaceae) (PARC/2013/2088), seeds of *Trigonella foenum-graecum* L. (Papilionaceae) (PARC/2013/2087), fruits of *Momordica charantia* (Cucurbitaceae) (PARC/2013/2101), were collected from in and around Tirupathi, India in the month of January 2013 and it was identified and authenticated. The taxonomical identification and authentication of the plant was done by Dr. P. Jayaraman, Director, National Institute of Herbal Medicine, Plant Anatomy Research Centre, Chennai. The voucher specimen is preserved in laboratory, Department of Pharmacognosy, Sree Vidyanikethan College of Pharmacy for further reference. Rosemary oil from *Rosmarinus officinalis* L (Lamiaceae) directly purchased from a grocery store.

Preparation of poly herbal extract

The leaves of *Gymnema sylvestre* R.Br. (Asclepidaceae), *Psidium guajava* (Myrtaceae), bulbs of *Allium sativum* L (Liliaceae), *Acalypha indica* (Euphorbiaceae), rhizomes of *Curcuma longa* (Zingiberaceae), *Zingiber officinale* (Euphorbiaceae), seeds of *Trigonella foenum-graecum* L. (Papilionaceae), fruits of *Momordica charantia* (Cucurbitaceae) were collected, washed and shade dried. After

complete drying, they were powdered and passed through a 60 mesh sieve and stored in air tight container. Dried powdered drugs were used to prepare extract. About 50 g of shade dried powders of each plant materials were mixed and extracted with alcohol by soxhlet extraction. The solvent was evaporated and accurate weight of the extract was noted. Extractive value (in %) was calculated with reference to air dried drug.

Preliminary Phytochemical Screening

The Preliminary phytochemical studies were conducted for above poly herbal extract to find out the presence of various phytoconstituents such as carbohydrates, alkaloids, glycosides, flavonoids, phenolic compounds and tannins as per the standard procedures [25].

3. ANTI MICROBIAL ACTIVITY:

The screening of Anti-microbial efficacy of the formulated Poly herbal extract was performed on various micro organisms by using modified agar well diffusion method [26]. Four sterile petri

plates were taken for testing the anti microbial activity against different microorganisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis*. Nutrient broth was prepared and inoculated with the micro organisms and incubated at 37⁰ for 24 hours. The next day nutrient agar medium was prepared and allowed for solidification. After solidification the microorganisms were inoculated into the nutrient agar media and two cavities were made by using sterile borer at equidistance of each plate. The two cavities were filled with poly herbal extract and standard Streptomycin (marketed antibiotic) and incubated at 37⁰C for 24hrs. Next day the Petri plates were observed for the formation of zone of inhibition. The antimicrobial activity was estimated by measuring the diameter of zones of inhibition (in mm).

4. RESULTS:

Preliminary Phytochemical screening

Table 1: Preliminary Phytochemical Analysis of ethanolic extract

Plant name	Carbohydrates	Alkaloids	Glycosides	Tannins	Flavonoids	Polyphenols	Saponins
<i>Acalypha indica</i>	+	-	-	+	+	-	-
<i>Allium sativum</i>	+	+	+	-	+	+	-
<i>Curcuma longa</i>	+	-	+	-	+	-	+
<i>Gymnema sylvestre</i>	+	+	+	+	+	+	-
<i>Momordica charantia</i>	-	+	+	-	+	+	-
<i>Psidium guava</i>	+	+	+	+	+	+	+
<i>Rosmarinus officinalis</i>	-	+	-	+	+	+	+
<i>Trigonella foenum-graecum</i>	+	+	+	+	+	+	+
<i>Zingiber officinale</i>	+	+	+	+	+	-	+

Antimicrobial activity

The results of modified agar well diffusion method (Table 1) showed that the developed poly herbal extract inhibitory effect on the microorganisms responsible for the diabetic wound infection. The activity of the poly herbal extract has been comparable to that of marketed antibiotic.

The diameter of zones of inhibitions is given in the table no.1 and the zones of inhibition by poly herbal extract against the microorganisms responsible for diabetic wound infection such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis* were showed in the fig numbers 1, 2, 3, 4 respectively.

Table 2: Anti-microbial sensitivity result of the formulation

Microorganisms	Zone of inhibition (mm)	
	Poly Herbal extract	Marketed formulation
<i>Staphylococcus aureus</i>	12mm	18mm
<i>Pseudomonas aeruginosa</i>	14mm	18mm
<i>Escherichia coli</i>	13mm	18mm
<i>Bacillus subtilis</i>	14mm	18mm



Fig 1



Fig 2



Fig 3



Fig 4

5. DISCUSSION:

Many medicinal plants extracts have been known to possess antimicrobial activity and are used for the treatment of microbial infections due to the presence of certain chemical constituents. Herbal remedies are considered as safer than the allopathic medicines due to side effects like local irritation, contact allergy, photosensitivity, itching, pruritus skin peeling, redness of the skin etc., and the preliminary phytochemical investigation revealed the presence of various chemical constituents in the poly herbal ethanolic extract was reported in the Table 1.

6. CONCLUSION:

The developed poly herbal extract was showed significant antimicrobial activity against the microorganisms responsible for diabetic wound infection. In majority of traditional systems of medicine, diabetes is better managed by the combination of herbs (Polyherbal) instead of single herb because of synergism and less side effects. When compared to synthetic medicines herbal remedies are more acceptable as they are safer with fewer side effects than the synthetic ones.

In the present study poly herbal ethanolic extract showed excellent antimicrobial activity which may be due to the presence of phytoconstituents such as tannins and phenolic compounds. So this in vitro antimicrobial study demonstrated that folk medicine can be as effective as modern medicine to kill the pathogenic microorganisms.

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