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INVESTIGATION OF *IN VITRO* ANTIMICROBIAL AND ANALGESIC ACTIVITY OF POLYHERBAL EXTRACT

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ABSTRACT

Increase in prevalence of side effects of conventional therapies has increased the need to search for an alternative therapy of natural origin. A Polyherbal extract was prepared by mixing extracts isolated from *Piper bettle, Elettaria cardamomum* and *Eugenia caryophyllata.* The extract was investigated for its anti microbial and antinociceptive properties. The combined extract produced pronounced antibacterial and antifungal properties against *Pseudomonas aeruginosa, E coli, Bacillus subtilis, Staphylococcus aureus and Aspergillus niger.* The combined extract also produced comparable anti nociceptive properties tested on two group's albino mice.

Keywords: Polyherbal extract, anti microbial, *Pseudomonas aeruginosa* and anti nociceptive activity

INTRODUCTION

Practice of traditional folk medicine since years has surpassed the side effects of conventional medicine. Combination of poly herbal extract is always a trump card compared to usage of single medicine. Betel (*Piper bettle*) belonging to the family Piperaceae known for its multiple benefits like anti inflammatory, antiproliferative, immunomodulatory activities etc. Cardamom (*Elettaria cardamomum* also called as "Queen of Spices" belongs to the family Zingiberaceae. It produces pods having around 20 seeds with abundant medicinal properties like antispasmodic, aphrodisiac, expectorant etc. **[1, 2]**. Clove (*Eugenia caryophyllata)* belonging to the

family mrytaceae an aromatic flower bud contains 15-20% of essential oil Eugenol known for its anodyne, aphrodisiac, carminative and anti inflammatory properties **[3]**. The advantage of polyherbal formulation include synergistic effect, mutual antagonistic effects **[4]**. The present study aims to evaluate *in vitro* antimicrobial and antinociceptive action of a polyherbal extract prepared from Betel leaves, cardamom seeds and clove buds.

MATERIALS AND METHODS

Betel leaves, cardamom pods and clove buds are collected, cleaned thoroughly and dried under shade. They are powdered and extracted for its principal constituents. The extracts were combined in the ratio of 1:1:1 and the combined polyherbal extract were evaluated for antimicrobial activity and anti nociceptive activity.

Extraction of active constituents from betel leaves

1kg of dry powder betel leaves were macerated twice with 96% ethanol for 72hours at room temperature (28-30^0 C) The extract was filtered and the filtrate is evaporated to dry with rotary evaporator at 40^{0}C.

Extraction of active constituents from Cardamon seeds and clove buds

Dried cardamom seeds (50g) were grounded thoroughly and steam distilled to yield essential oil. The collected oil was separated using dichloro methane in a separating funnel.

Phytochemical Screening of active constituents

Test for Eugenol

1. To 2ml of extract few ml of sudan III was added
2. To 2ml of extract few ml of freshly prepared Ferric chloride solution was added
3. To 2ml of extract add few ml of KOH solution.

Test for Cineole

To 2ml of extract few ml of resorcinol (50%w/v) was added.

Test for Alkaloid

Mayer's Test

To the 2ml of extract, few ml of mayers reagent was added cream colour precipitate was obtained

Wagner's Test

To 2ml of extract, few ml of wagner's reagent was added, reddish brown colour ppt was obtained

Test for Tannins

To 2ml of extract, few ml of ferric chloride is added green colour

Test for Glycosides

Keller Killani test

To 2ml of extract, few ml of glacial acetic acid, 1 drop of 15% Ferric chloride and few ml of Conc. H_2SO_4 was added, reddish

brown colour was observed at the junction **[5]**

Determination of Anti Microbial activity

Anti microbial activity of individual extracts as well as combined extracts was determined by agar well diffusion method. Sterile Muller-Hinton agar was plated in the petri plates, inoculated with test organisms. Well were bored using sterile borer and into the wells , 1000 µg / ml extract and Standard antibiotic of 1000 µg / ml were added incubated for 48 hrs, at 37°C. Anti microbial activity spectrum was determined by taking four bacteria and one fungi namely *Pseudomonas aeruginosa, E coli, Bacillus subtilis, Staphylococcus aureus and Aspergillus niger* **[6, 7]**.

Determination of anti- nociceptive effect by hot plate procedure

Ant nociceptive effect of combined polyherbal extract was investigated by hot plate test using 2groups of ten albino mice. Reaction time was recorded at 0, 10, 15 and 30min time. Latency time was measured and compared to the standard treated group **[8]**. Percentage anti nociceptive activity was determined by using the formula:

$$PAA = ((L_a - L_b)/L_b) \times 100$$

L_a =Latency time after treatment with extract

L_b=Latency time before treatment with extract

RESULTS AND DISCUSSION

Betel leaves, Cardamom pods and Clove buds were extracted by their respective procedures and screened for their phytochemical constituents and results of phytochemical screening was tabulated in the **Table 3.1**. The results indicates the presence of Eugenol ,alkaloids and glycosides in betel leaf extract, presence of cineole in cardamom extract and Eugenol in clove extract.

Determination of anti microbial activity of extracts

A blended extract was prepared by combining extracts of Betel leaves, cardamom seeds and clove extract and was evaluated for antimicrobial and anti nociceptive activities. Anti microbial activity of the extracts were determined by agar diffusion method and the results were tabulated in the **Table 3.2** which indicates that all the extracts have effective anti fungal properties as well as anti bacterial properties inhibiting both gram positive and gram negative bacteria indistinguishable to antibiotic preparation .The results also indicate that combination of extracts gives a syngergistic antimicrobial action with mutual antagonistic properties.

Determination of anti nociceptive effect

Antinociceptive effect of the combined extracts were studied by hot plate method , percentage latency was calculated at the reaction 0min,10min and 15min. Percentage latency of the combined extract treated for two groups was inconsiderably less than the standard **(Table 3.3)**.

Table 3.1: Phytochemical screening of various extracts

Tests	Betel leaf extract	Cardamom extract	Clove extract
Test for Eugenol	++	_	++
Test for Cineole	-	++	-
Test for Alkaloids			
Mayer's tests	++	_	_
Wagner's test	++	_	_
Tannins test	++	_	_
Glycoside test	++	_	_

Table 3.2: Determination of anti microbial activity of extracts

Extract (1000 µg/ml)	*Pseudomonas aeruginosa*	*E.coli*	*Bacillus subtilis*	*Staphylococcus aureus*	*Aspergillus niger*	Cefataxime (1000 µg / ml) (Standard)
	Zone of inhibition(cm)					
Clove Extract	3.2	2	2.5	3.5	No growth	3.6
Cardamom extract	1.2	2.2	2	3.0	No growth	3.4
Beetle leaf extracy	3.5	2.1	1.5	2.0	No growth	3.4
Combined extract	3.7	2.8	2.7	4.0	No growth	3.5

Table 3 .3: Determination of anti nociceptive effect of combined extract

	Percentage latency		
Reaction Time	0 min	10min	15min
Group 1	25.0	65.0	80.0
Group 2	20.0	56.0	72.0
Standard	20.0	95.0	96.0

CONCLUSION

Combined poly herbal extracts produced a pronounced antimicrobial and antinociceptive action compared to individual extracts comparable to conventional therapy. The upswing of side effects and multi drug resistance of conventional drug therapy has triggered the research towards traditional medicine. Combination of folk medicines appears to be more advantageous than single drugs due to their synergistic action. Careful selection of folk medicines together with optimizing their concentration would be more beneficial in treating many diseases.

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