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Anti-Microbial Potential of two Traditional Medicinal Plants

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ABSTRACT

Background: Consumers as well as scientific community all over the world, including developed countries are becoming disillusioned with modern health care and are seeking alternatives. In current scenario, it is a better option to capture the wisdom of traditional healing systems, and this would lead to basic scientific investigation on the medicinal plants, to exploit the natural bioactive sources for safe and easily approachable natural therapeutic agent. **Objective:** The main objective of the study is to find out anti-microbial potential of two important traditional medicinal plants, Pittosporum crassifolium and Citrullus colocynthis Linn. **Study Design:** Experimental study. **Settings:** Different departments of school of Pharmacy, Pathology and Biochemistry of University Medical & Dental College/ The University of Faisalabad, Faisalabad Pakistan. **Duration:** October 2017 to February 2018. **Methodology:** Antibiotic assay is performed by using the disc diffusion methods. The extracts of plants are prepared and from theses sensitivity disc are prepared. Standard size inoculum is also prepared. Then by using the standard disc diffusion method, the zones of inhibitions produced by plant extracts are measured and recorded in mm and are compared with positive & negative controls. **Results:** Extracts of two medicinal plants (Pittosporum crassifolium and Citrullus colocynthis Linn) were assessed for their antimicrobial activities against medically significant pathogenic bacterial strains capable of causing wide varieties of ailments (Infectious diseases). All plant extracts tested, showed significant antimicrobial activity against test organism (Gram negative and Gram positive), with zone of inhibition 8.33±0.33l to 19.00±2.67. Ethanol extracts prepared by soxhlet apparatus showed better results compared with extracts prepared by simple maceration process. Furthermore, over all ethanol extract showed better results compared with chloroform and aqueous extracts. **Conclusion:** This study revealed the presence of natural bioactive compounds in both plants with highly significant broad spectrum antibacterial potential against all test organisms.

Keywords: *Antimicrobial potential, Natural therapeutic agents, Natural bioactive source, Soxhlet apparatus, Traditional Medicinal plants.*

INTRODUCTION

Some microorganisms are extremely pathogenic causing serious ailments in human. The discovery of antibiotics to treat these pathogens marked a revolution in the 20th century. Unfortunately, due to misuse of antibiotics certain strains of microorganisms are developing resistance against antibiotics by blocking action of antibiotic or changing their targets or ability to penetrate the cells.[1]

Further antibiotics are posing some serious threats.[2] Antibiotics can bring significant changes in intestinal micro ecology resulting in overgrowth and colonization of pathogens.[3]

Control of multi drug resistance microorganisms and management of ailments caused by them is emerging threatening problem worldwide[4]. Substantial rise in emergence of multi drug resistance microbes is becoming the main factor of failure of managing/treating the microbial diseases,[5] and society is facing one of the most challenging public health dilemmas.[6]

Excessive and indiscriminate use of allopathic medicine resulted in serious changes in gut micro biota.[7] Over the last decade, some of the microorganisms have emerged as the most problematic nosocomial pathogens, multi drug resistance clones of which have been disseminative worldwide.[8]

It is estimated that about 70% of bacteria that caused ailments in hospitals, become in sensitive to at least one of the drugs currently prescribed for treatment[9]. Continuous spread of multi drug resistance pathogens, increasing cost of drug regimens and increasing side effects of current allopathic medicine, the scenario has paved the way for new and reemerging infectious diseases worldwide.[10]

Medicinal plants have been used in preparations of herbal medicines since ancient time. The use of plant extracts and phyto products is continuously regaining popularity due to their availability, cost effectiveness, natural source of origin and ecofriendly behavior. Further, they have shown clinically significant antiviral, antifungal, and antibacterial activities.[11]

Some Pakistani medicinal plants have shown significant in vitro antibacterial activity.[12] Current research studies are also proving that plants are still recognized as bedrock for modern medicines to treat infectious diseases [13]; and several chemical compound used in modern medicine, which were derived from plant sources include quinine, digoxin, aspirin, ephedrine, atropine and colchicine;[14] so medicinal plants are said to be the future source of new drugs.[15,16]

The world over researcher are trying to tape/probe the plant kingdom as natural source for drugs of desirable efficacy and safety profile. So, in the existing scenario some medicine plants which are used in traditional medicines for different ailments in Sub continent, are evaluated for their antimicrobial activity. With reference to the modern allopathic medicine, these plants are not adequately investigated and evaluated except Citrulluscolocynthis (Tumma) which is to some extent investigated for some of its medicinal properties. Both plants are tested against most common problematic pathogens are selected as test organisms; *Salmonella Typhi, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Echerichia coli, Staphylococcus aureous.*

Objectives: (1) To investigate the antimicrobial activities of commonly used medicinal plants (in-traditional medicine) in this area (Punjab) of the Subcontinent. (2) To explore the natural bioactive source for natural therapeutic agents as an alternative to problematic antibiotics.

METHODOLOGY

Study Design: Experimental study.

Settings: Different departments of school of Pharmacy, Pathology and Biochemistry of University Medical & Dental College / The University of Faisalabad, Faisalabad Pakistan.

Duration: October 2017 to February 2018.

Sample Technique: Soxlet Apparatus, Disc diffusion method.

Sample Size: n:2

Inclusion Criteria and Exclusion Criteria: Due to increased resistance of bacteria against antibiotics, antimicrobial activity of natural products with a high level of safety is of increasing interest. In this study, Pittosporum crassifolium and Citrullus colocynthis) have been included because their antimicrobial activity against different bacteria is required to be further explored for safe use and reduction of microbial resistance. However other species of Pittosporum and Citrullus have been excluded from the study as sufficient information is available about their antimicrobial potential.

Data Collection Procedure: The plants include Pittosporum crassifolium and Citrullus colocynthis Linn. These plants were collected from the market and their identification were got confirmed from a botanist. Both plants were thoroughly washed in distilled water and dried in shade at room temperature for three days. After drying, all plants were ground by using electric grinder. 30 grams of each plant powder was mixed with 100ml of ethanol, chloroform and distal water separately. Extracts were prepared with the help of Soxhlet apparatus as well as simple maceration process. Then 10 µl of each extracts impregnated into empty sterilized antibiotic disc.

Preparation of Master Culture: Antimicrobial activity of plants were investigated against six organisms i.e. gram positive batcteria Staphyloccocus aureous and gram negative bacteria Salmonella typhi, Pseudomonas aeruginosa, E. coli, proteus vulgaris and klebsiella pneumonia. The pure culture of microorganisms were obtained from the diagnostic center of (Madinah Teaching Hospital) Faisalabad.

The bacterial isolates were sub-cultured in agar slants in order to obtain pure Master Culture regularly (every 30 days). From this fresh culture standard size (10^8 to 10^9 CFU per ml) inoculums is prepared and used.

Table 1: Botanical information of medicinal plants used

Botanical name of plants	Common name (Urdu)	Common name (English)	Family	Parts used
Pittosporum crassifolium	Karo	Stiff leaf cheese wood	Pittosporaceae	Whole Plant
Citrullus colocynthis Linn	Tumma	Indrayan	Cucurbitaceae	Fruit with seeds

Antimicrobial Bioassay: Discs prepared were placed aseptically over the standard size inoculums on the nutrient agar plates along with positive and negative controls and incubated at 37^0c for 24 hours. All treated petri plates were immediately placed in incubator at 37^0c. Sterile, blank paper discs impregnated with only sterile water, ethanol and chloroform were used as negative control each time. Standard Ciprofloxacin was used as positive control for comparison of antibacterial activity.

After 24 hours incubation, all the plates having discs for alcoholic extracts, water extracts and chloroform extracts were observed for their zone of inhibition. The zone of inhibition around the discs were measured by venire caliper. The data of zone of inhibition of two plants having three types of solvent extracts (water, chloroform and ethanol) and a control against six bacterial pathogens was recorded with three times repeats to confirm the reproducible results of plant extracts. Since all the observations in negative control were zero therefore data in negative control was not used for statistical analysis.

Data Analysis: Data was analyzed by two-way analysis of variance. The data collected for each experiment are subject to statistical analysis. Analysis of variance (SPSS) was used. The data were presented in tabulated form or graphical form.

RESULTS

The present study assessed/investigated the anti-biogram of two medicine plants. Extracts were prepared by two methods; Soxhlet and maceration, using three different solvents (ethanol, chloroform and distilled water). Results are presented in the table # 2-5.

Regarding the methods; Soxhlet method proved more effective.

Among the solvents (for these plants), ethanol showed better extraction power as an organic solvent for the plant Investigated

Plants:

- Ethanol extracts of Picrorhiza Kurroa showed zone of inhibition ranging from S.typhi (18mm),Klebsiella (18mm),E.coli (16mm), protease vulganis (15mm), S.aureous (13mm)and pseudomonas aeruginosa (11mm). Among these organisms S.typhi and Klebsiella showed higher zone of inhibition.
- Ethanol extracts of Citrullus Colocynthis Linn showed zone of inhibition ranging from Klebsiella (11mm), pseudomonas aeruginosa (11mm), S.aureous (11mm), S.typhi (10mm), protease vulganis (10mm)and E.coli (9mm). Among these organisms Klebsiella, pseudomonas aeruginosa and S.aureous showed higher zone of inhibition.
- Control Drug (Ciproxin) showed zone of inhibition ranging from S.thphi (28mm), E.coli (26mm), S.aureous (24mm), pseudomonas aeruginosa (24mm), protease vulganis (22mm), among these tested organisms S.typhi showed higher zone of inhibition.
- Bacteria: All bacteria tested proved susceptible to the plant extracts evaluated.

Table 2: Analysis of variance table

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	P-value
Extract (E)	4	152.16	38.04	56.13**	0.000
Plant (P)	2	12023.67	6011.84	8869.92**	0.000
Bacteria (B)	5	258.56	51.71	76.30**	0.000
E x P	8	184.10	23.01	33.95**	0.000
E x B	20	75.88	3.79	5.60**	0.000
P x B	10	274.68	27.47	40.53**	0.000
E x P x B	40	129.32	3.23	4.77**	0.000
Error	180	122.00	0.68		
Total	269	13220.39			

*NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01)*

Table 3: Extract x Plant interaction mean±SE

Extract (Method)	Plant			Mean
	Standard drug (Ciprofloxacin)	Karo	Tumma	
Ethanol (Soxhlet)	24.83±0.52a	15.22±0.64b	10.33±0.23de	16.80±0.87A
Ethanol (Maceration)	24.78±0.59a	12.44±0.36c	9.28±0.18fg	15.50±0.95B
Chloroform (Soxhlet)	24.56±0.53a	11.17±0.43d	9.61±0.37efg	15.11±0.96BC
Chloroform (Maceration)	24.72±0.46a	9.83±0.35ef	9.72±0.48efg	14.76±1.00C
Distilled Water (Maceration)	24.78±0.50a	10.78±0.35d	8.83±0.20g	14.80±1.00C
Mean	24.73±0.23A	11.89±0.28B	9.56±0.15C	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 4: Bacteria x Plant interaction mean±SE

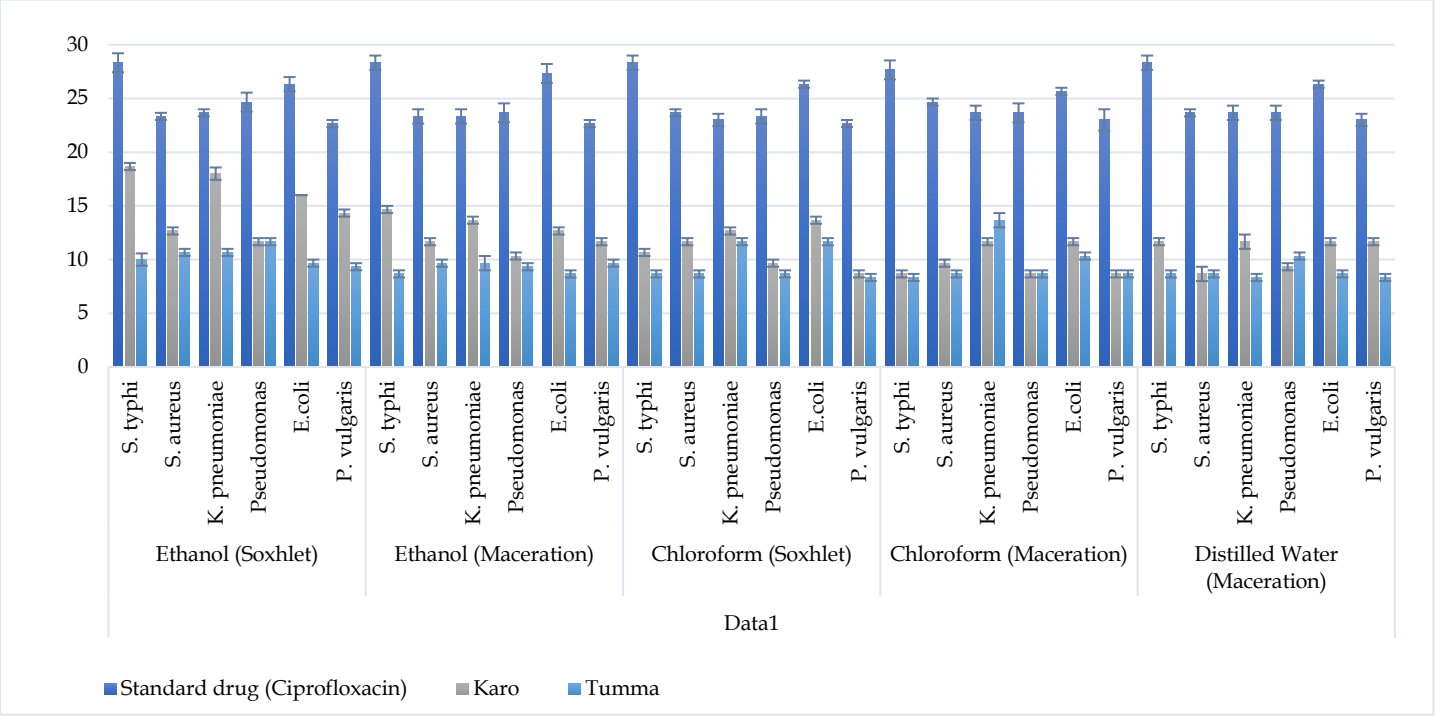
Bacteria	Plant			Mean
	Standard drug (Ciprofloxacin)	Karo	Tumma	
Salmonella typhi	28.20±0.30a	12.87±0.94d	8.87±0.22i	16.64±1.30A
Staphylococcus aureus	23.73±0.21c	10.87±0.42ef	9.27±0.25hi	14.62±0.99C
Klebsiellapneumoniae	23.47±0.24c	13.53±0.65d	10.80±0.52efg	15.93±0.87B
Pseudomonas	23.80±0.33c	9.93±0.30fgh	9.73±0.33hi	14.49±1.01C
E.coli	26.40±0.25b	13.13±0.45d	9.80±0.33ghi	16.44±1.10A
Protease vulgaris	22.80±0.22c	11.00±0.59e	8.87±0.19i	14.22±0.95C

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 5: Extract (Method) x Bacteria x Plant interaction mean±SE

Extract (Method)	Bacteria	Plant			Mean
		Standard drug (Ciprofloxacin)	Karo	Tumma	(Extract x Bacteria)
Ethanol (Soxhlet)	**Salmonella typhi**	28.33±0.88a	18.67±0.33f	10.00±0.58jkl	19.00±2.67A
	Staphylococcus aureus	23.33±0.33de	12.67±0.33hij	10.67±0.33jkl	15.56±1.97E-H
	Klebsiellapneumoniae	23.67±0.33cde	18.00±0.58f	10.67±0.33jkl	17.44±1.89B
	Pseudomonas	24.67±0.88b-e	11.67±0.33ijk	11.67±0.33ijk	16.00±2.19B-F
	E.coli	26.33±0.67abc	16.00±0.00fg	9.67±0.33kl	17.33±2.44BC
	Protease vulgaris	22.67±0.33e	14.33±0.33ghi	9.33±0.33kl	15.44±1.95E-H
Ethanol (Maceration)	**Salmonella typhi**	28.33±0.67a	14.67±0.33gh	8.67±0.33l	17.22±2.92BCD
	Staphylococcus aureus	23.33±0.67de	11.67±0.33ijk	9.67±0.33kl	14.89±2.14E-I
	Klebsiellapneumoniae	23.33±0.67de	13.67±0.33ghi	9.67±0.67kl	15.56±2.05E-H
	Pseudomonas	23.67±0.88cde	10.33±0.33jkl	9.33±0.33kl	14.44±2.33G-J
	E.coli	27.33±0.88ab	12.67±0.33hij	8.67±0.33l	16.22±2.85B-E
	Protease vulgaris	22.67±0.33e	11.67±0.33ijk	9.67±0.33kl	14.67±2.03F-J
Chloroform (Soxhlet)	**Salmonella typhi**	28.33±0.67a	10.67±0.33jkl	8.67±0.33l	15.89±3.13C-G
	Staphylococcus aureus	23.67±0.33cde	11.67±0.33ijk	8.67±0.33l	14.67±2.30F-J
	Klebsiellapneumoniae	23.00±0.58de	12.67±0.33hij	11.67±0.33ijk	15.78±1.82D-H
	Pseudomonas	23.33±0.67de	9.67±0.33kl	8.67±0.33l	13.89±2.38IJ
	E.coli	26.33±0.33abc	13.67±0.33ghi	11.67±0.33ijk	17.22±2.30BCD
	Protease vulgaris	22.67±0.33e	8.67±0.33l	8.33±0.33l	13.22±2.37J
Chloroform (Maceration)	**Salmonella typhi**	27.67±0.88a	8.67±0.33l	8.33±0.33l	14.89±3.21E-I
	Staphylococcus aureus	24.67±0.33b-e	9.67±0.33kl	8.67±0.33l	14.33±2.59HIJ
	Klebsiellapneumoniae	23.67±0.67cde	11.67±0.33ijk	13.67±0.67ghi	16.33±1.88B-E
	Pseudomonas	23.67±0.88cde	8.67±0.33l	8.67±0.33l	13.67±2.52IJ
	E.coli	25.67±0.33a-d	11.67±0.33ijk	10.33±0.33jkl	15.89±2.46C-G
	Protease vulgaris	23.00±1.00de	8.67±0.33l	8.67±0.33l	13.44±2.41IJ
Distilled Water (Maceration)	**Salmonella typhi**	28.33±0.67a	11.67±0.33ijk	8.67±0.33l	16.22±3.07B-E
	Staphylococcus aureus	23.67±0.33cde	8.67±0.67l	8.67±0.33l	13.67±2.51IJ
	Klebsiellapneumoniae	23.67±0.67cde	11.67±0.67ijk	8.33±0.33l	14.56±2.35F-J
	Pseudomonas	23.67±0.67cde	9.33±0.33kl	10.33±0.33jkl	14.44±2.32G-J
	E.coli	26.33±0.33abc	11.67±0.33ijk	8.67±0.33l	15.56±2.73E-H
	Protease vulgaris	23.00±0.58de	11.67±0.33ijk	8.33±0.33l	14.33±2.23HIJ

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.



DISCUSSION

Since, the traditional healers use plant products (extracts) for various diseases, is based on empirical knowledge, the scientific research studies of all these herbal drugs are highly desirable regarding their efficacy, safety profile and scientific validation.

Traditional medicine knowledge and its application may help in the development of indigenes medicines (from traditional medicine plants), that might improve the quality of health care system/ delivery, enhance economic benefits and optimize functioning of eco systems of the respective area/country.

(i) Methods: Soxhlet method proved to be better because different conditioning factors (temperature, pressure and pH etc.) are properly/effectively controlled by this apparatus.

(ii) Solvents: In present study, the ethanol proved to be better extractor, for these selected plants, as were the cases of previous studies in number of other different plants.[17,18,19,20]

But in other studies, different extracting solvents proved to be more effective for different plants. Methanol proved to be a bette extractor for psidiumguajava, nigella satice, sitrussinensis, calerianjutamansi and cucurbita papa.[13] Other extractors: Chloroform for foeniculumvalgare Mill,[21] distilled water for LawsuniainternisLimm,[22] and petroleum ether for Trigonella foenum-graecum[14] proved more effective than other solvents. Differential extraction ability/power of solvents may be because of different solubility of different active principles of different plants in different solvents. So, is appears to be related with differential solubility of active principles of plants for different solvents. It is thus concluded that in preparation of extracts, the solvents used are very important factor. So, different solvents should be probed to find out the best solvent for particular plant.

(iii) Plants: Generally a plant is consider to be active/effective against pathogenic microbes when the zone of inhibition is greater than 6mm.[23] Results showed/indicated that both medicinal plants extracts tested, significantly inhibited the growth of all tested pathogen bacteria at varying degrees. The maximum zone of inhibition (18mm) was fond with ethanol extracts of Pittosporum crassifolium (against S. typhi) and Citrullus colocynthis Linn (against S. typhi, pseudomonas aeruginosa, and protease vulgains) the minimum zone of inhibition (8.5± mm) was found with chloroform extracts of Citrullus colocynthis Linn (against S.typhi).

Results obtained from in vitro antimicrobial activity showed that all two plant extracts exhibited a substantial/significant inhibitory effect against the all six test bacteria. Ethanol extracts of all plants proved superior in suppressing the bacterial growth, followed by water and chloroform extracts. Ethanol extracts exhibited promising antibacterial activity against all six tested bacteria (both gram negative and positive), may be due to presence of phenols and flavonoids (active principles/ingredients) which are better extracted with ethanol.[24] The lesser activity seen with other solvents may be due to low solubility of active sinsitivents/ principles in the other solvents.[24]

Varying degrees of antimicrobial activities may be due to different;

(i) Active principles within the different plants

(ii) Solubility of active principle in different solvents

(iii) Conditioning factor (temperature, pressure and pH tec.) during processing

In present study, both plant extracts revealed significant antimicrobial activity against both Gram negative and Gram-positive microbes, proving their broad-spectrum potential. Regarding the degree of antimicrobial activity of plants; Pittosporum crassifolium was at the top, followed by Citrullus Colocynthis Linn and then the Embelliarobusta, and forth position was that of Citrullus colocynthislinn.

Studies all over the world are acknowledging the different properties/activities of medicinal plants, and it seems that very soon the plant kingdom is going to be the Veritable Universal Natural Source of all type of desired drugs.

(iv) Bacteria: Regarding the susceptibility; S.typhi proved to be most sensitive organism to reference drug and also against the plan extracts. This is followed by the in a descending order, Klebsuella pneumonia. Proteus vulgaris, Psedudomonasaeruginosa,

Echerichia coli, and lasty Staphylococcus aureous.

(v) Overall Impact: Since, the use of plant products (extracts) for various diseases by traditional healers, is based on empirical knowledge, therefore, the scientific studies of all these herbal drugs are highly desirable regarding their efficacy, safety profile and scientific validation.

So, in the existing scenario, the traditional medicine knowledge and its application may help in the development of indigenes medicines (from traditional medicine plants), that might improve the quality of health care system/delivery, enhance economic benefits and optimize functioning of eco system of the respective are /country.

CONCLUSION

Crude extracts of plants (under investigation) significantly inhibited common medically important isolates (Gram positive & Gram negative), proving that these plants might have potential as an alternate source of broad spectrum antimicrobial agents effective against multi drug resistant (MDR) pathogens including life-threatening S.typhi. So, there is need to isolate the bioactive components (bio-principles) that are responsible for the ethno-pharmacological properties of these medicinal plant extracts. This might be accomplished after proper purification, quality chemotherapeutic index and pharmaceutical analysis.

LIMITATIONS

The plants extract still required to be subjected to animal and human studies after analyzing in vitro antimicrobial potential to regulate their efficacy in whole-organism systems, specifically including toxicity studies as well as an investigation of their effects on normal microbiota.

SUGGESTIONS / RECOMMENDATIONS

There is need to isolate the bioactive components that are responsible for the ethno-pharmacological properties of this medicinal plants. This is might be accomplished after proper purification, quality chemotherapeutic index and pharmaceutical analysis of this plant extract.

CONFLICT OF INTEREST / DISCLOSURE

None.

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