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RESEARCH ARTICLE

In vitro biological activities of Selected Medicinal Plant Extracts

Satheesh Kumar C.S[1], Aleykytty N[2], Jolly C.I[3] Jayakar B[4]*

[1]Research Scholar Vinayaka Mission University, Salem, Tamil Nadu 636 308
[2]Pushpagiri College of Pharmacy, Medicity, Thiruvalla,Kerala 689 101
[3]Kerala Ayurveda pharma, Athani, Aluva, Ernakulam, Kerala 683 585
[4]Vinayaga Mission's College of Pharmacy, Salem, Tamil Nadu, 636 308

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Abstract

This present study focuses on the *in vitro* biological activities of a polyherbal formulation composed of nine medicinal plants which were selected based on the traditional use in medicines. Hydroalcoholic extracts were prepared and evaluated for their *in vitro* efficacy by different models to shortlist for the final formulation. The extracts were tested for their antimicrobial, antioxidant and anti inflammatory activities. Among these, *Andrographis paniculata, Coleus aromaticus*, *Tinospora cordifolia, Zingiber officianale* and *Piper nigrum* extracts exhibited promising inhibitory activities against selected microorganisms. Extracts from *Andrographis paniculata* and *Tinospora cordifolia* exhibited significant scavenging activity against DPPH and NO free radicals. These two extracts exhibited strong anti-inflammatory response by inhibiting the LPS induced TNF in RAW cells. Among the tested extracts, five extracts from AP (*Andrographis paniculata)*, CA(, *Coleus aromaticus)*, TC(*Tinospora cordifolia)*, ZO(*Zingiber officianale)* and PN(*Piper nigrum*) were found to have promising antimicrobial, antioxidant and anti-inflammatory properties.

INTRODUCTION

Terrestrial plants have played a dominant therapeutic role in the treatment of human ailments from time immemorial. Plants have been used as a primary source of medicine for millennia, with the earliest recorded uses found in Babylon circa 1770 B.C. in the code of Hammurabhi and in ancient Egypt circa 1550 B.C [1]. In India, reference to the system of Ayurveda dates back to 900 B.C. Till last century, medical practice depended largely on the use of plants and the knowledge about the use of herbal medicines was mainly based on age-long empirical experience communicated from one generation to the next which formed the basis of many traditional medicinal systems including Ayurveda in India. It is common knowledge in many of today's societies that plants or their extracts continue to provide effective treatment for diseases of all kinds, as they have been for centuries. Ayurvedic medicine in India and traditional Chinese medicine both depend to a large extent on the use of precise plant-based prescriptions for the treatment of specific disorders. Similar contemporary practices are found in many regions, for example in other parts of Asia, South America, Africa, the Middle East and Russia [2]. A major lacuna in Ayurveda is the lack of drug standardization, information and quality control. Most of the Ayurvedic medicines are in the form of crude extracts which are a mixture of several ingredients and the active principles when isolated individually fail to give desired activity. This implies that the activity of the extract is the synergistic effect of its various components. In the absence of pharmacopoeia data on the various plant extracts, it is not possible to isolate or standardize the active contents having the desired effects [3]. The scientific study of traditional medicines, derivation of drugs through bio prospecting and systematic conservation of the concerned medicinal plants are thus of great importance. Traditional systems of medicine continue to be widely practised on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and

▼ To whom correspondence should be addressed:
Mr. Satheesh Kumar C.S
E-Mail address: satheesh1010@gmail.com

development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Search for newer antipyretics, particularly herbal remedies has gained a momentum in recent times as available antipyretics like paracetamol, nemusilide etc, have toxic effects on various organs of the body. Moreover, these synthetic agents irreversibly inhibit COX-2 with high selectivity but are toxic to the hepatic cells, glomeruli, cortex of brain and heart muscles, whereas natural COX-2 inhibitors have lower selectivity with fewer side effects. A natural antipyretic agent with reduced or no toxicity is therefore essential [4]. Hence, keeping in view of the present scenario an attempt has been made to validate the folklore claims of selected medicinal plants *viz.Cyperus rotundus* (CR), *Piper nigrum* (PN), *Zingiber officinale* (ZO), *Andrographis paniculata* (AP), *Oldenlandia corymbosa* (OC), *Tinospora cordifolia* (TC), *Glycosmis pentaphyla* (GP), *Eupatorium Odoratum* (EO) and *Coleus aromaticus* (CO).

Materials and Methods

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), Sodium nitroprusside, naphthyl ethylene diamine dihydrochloride (NEDD), Lipo polysaccharide (LPS), Dexamethasone, Actinomycin- D and (4, 5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT) were procured from Sigma-Aldrich Co, St Louis, USA. Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Trypsin, Nutrient agar, Nutrient broth, Sabourd dextrose agar, Sabourd dextrose both, EDTA, Glucose and antibiotics were purchased from Hi-Media labs, Mumbai, India. Ethyl alcohol and Dimethyl Suphoxide (DMSO) were procured from SD Fine chemicals, Mumbai, India. All the chemicals used were of analytical grade.

Cell Culture and Microbial Strains

RAW 264.7 (Mouse macrophages) and L-929 (Mouse connective tissue) were procured from National Centre for Cell Sciences, Pune, India. Both the cell lines were maintained Dulbecco's Modified Eagle Medium (DMEM), where growth media supplemented with 10% Fetal bovine serum and cells were incubated at 37^0C with 5% CO_2 and 95% humidity unless specified.

Microbial cultures were procured from National Centre for Industrial Microorganisms, Pune, India. Cultures were maintained and sub-cultured regularly as per the supplier's instructions. Bacterial cultures were sub-cultured in Nutrient agar and fungal cultures were sub-cultured in sabourd dextrose agar.

Plant Material

Selected parts of plants, CR (rhizome), PN (dried fruit), ZO (rhizome), AP (whole plant), OC (aerial parts), TC (whole plant), GP (berries), EO (Aerial parts) and CA (leaf) were collected from different places of Kerala in natural condition and authenticated.

Preparation of Hydro Alcoholic Extracts

The raw materials were either powdered or chopped for size reduction and known quantity of this was charged in the extraction vessel. Ethyl alcohol-water mixture (70:30) was charged into the extractor, sufficient enough to fully soak the raw material (four times the quantity of raw material was used). The extractor was heated by applying jacket steam and a temperature of about 50°C maintained. The stirrer also in the extraction mass put on for fast and efficient extraction. After 3 hrs, the bottom drain valve of the extractor was opened and the total solvent was drained out. This was the first wash in the extraction process. Similarly three more washes were done and the solutions from the four washes are pooled, charged in an evaporator cum vacuum drier. The drying procedure was continued till the mass become pasty, vacuum of 600 mm Hg is applied in the vessel. At this condition, the mass got dried to small lumps, later discharged and powdered in pulverisor to get the final herbal extract.

Antimicrobial Activity

The extracts were screened for their antimicrobial activity against selected bacteria and fungi as described earlier to determine minimum inhibitory concentration (MIC) [5]. In this assay, a series of assay tubes were prepared with the final concentration of test substance ranging from 1000 to 3.9 g per ml. The tubes were incubated at 37^0C/28^0C for 24/48 hrs. The tubes were inspected visually to determine the growth of the organism as indicated by turbidity, the tubes in which the antibiotic was present in concentration sufficient to inhibit bacterial growth remain clear. In experimental terms the MIC was the concentration of the drug present in the last clear tube, i.e. in the tube having the lowest concentration in which growth was not observed.

Antioxidant Activity

The antioxidant potential of extracts was evaluated by DPPH scavenging[6] and Nitric oxide radical inhibition assays as described earlier [7]. Briefly in DPPH assay, the extract or standard solution (10µl) was added to 200µl of DPPH in methanol in a 96 well microtitre plate (Tarsons Product Ltd, India). After incubation at 37^0C for 30 min, the absorbance of each solution was

determined at 490nm using microplate reader (Biotek, USA). In nitric oxide radical inhibition assay, 2 ml sodium nitroprusside (10 mM), 0.5 ml phosphate buffer saline along with 0.5 ml of extract solution was incubated at 25ºC for 150 min. Then 1 ml of sulfanilic acid reagent (0.33% sulfanilic acid in 20% glacial acetic acid) was added to 0.5 ml of reaction mixture for 5 min to complete diazotization, subsequently. 1 ml naphthyl ethylene diamine dihydrochloride (NEDD) was added and allowed to stand for 30 min at 25ºC. The absorbance of these solutions was measured at 540nm.

Anti-Inflammatory Activity

In vitro anti-inflammatory activities of extract were determined by evaluating their TNF inhibition properties as described earlier [8] . In brief, RAW 264.7 cells(1×10^6/mL) were incubated with LPS (1g/mL) and Septilin at 5% and 2.5% or dexamethasone (100 M) in culture media with 2% FBS. Dexamethasone is a member of glucocorticoid and acts as an anti-inflammatory and immunosuppressant. Cells were incubated with and without extracts were incubated at 37°C for 4 hrs. The culture media were collected, centrifuged and stored at 20°C until used. L929 cells (1×10^5/mL) were seeded in 96-well plates and incubated overnight. The culture media from L929 cells were removed and conditioned media were added along with 25 L of actinomycin D (50 g/mL). The L929 cells pre-treated with actinomycin D appears sensitive to TNF-mediated Cytotoxicity and, it has been used to measure the presence of TNF in biological fluids and cell culture supernatants. The plates were incubated for additional 24 hrs. The TNF induced toxicity in L929 cells was determined by MTT assay [10].

Results

Antimicrobial Activity

Among the extracts tested, AP, CA, TC, ZO and PN were found to have promising antimicrobial activity with MIC values ranging from 62.5µg/ml to 1000µg/ml against different organisms [Table 1]. AP was found to be more potent among the tested extract with MIC values 62.5 µg/ml against *P. auriginosa* and 125 µg/ml against rest of the test organisms. Even the extracts of PN and CA also exhibited significant inhibitory properties. Other extracts exhibited poor inhibition against the microbial growth.

Antioxidant Activity

All the plant extracts showed antioxidant activity against both DPPH and nitric oxide radicals. Among the extracts tested, AP, CA, TC and ZO exhibited better antioxidant properties with low IC_{50} values against DPPH antioxidant properties could be expected to offer protection radical [Table 2]. Against NO radical, AP and TC exhibited significant activity with IC_{50} values 182.00 ± 0.81 and 186.43 ± 1.31µg/ml, respectively.

Anti-Inflammatory Activity

Results indicated that all the plant extracts have anti inflammatory properties [Table 3]. Among them plants, TC exhibited potent anti-inflammatory activity by inhibiting TNF significantly with 69.15% over control. AP, CA and PN exhibited significant anti inflammatory properties with TNF inhibition value 50.00, 47.56 and 56.50 percent, respectively.

Discussion

Despite the recent interest in molecular modelling, combinatorial chemistry, and other synthetic chemistry techniques by pharmaceutical companies and funding organizations, natural products, particularly medicinal plants, remains an important source of new drugs, new drug leads, and new chemical entities. It is evident that, natural products have played a vital role in drug discovery, by contributing a wide variety of phytochemical for the treatment of variety health disorders. In the present study, with an intention to develop anti-pyretic poly herbal formulation group of medicinal plants were selected. The selected plant extracts were evaluated for their antimicrobial, antioxidant and anti-inflammatory activities *in vitro*. Pyrexia or fever is caused as a secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. It is the body's natural defence to create an environment where infectious agent or damaged tissue cannot survive. Normally the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediators like interleukin 1beta, alpha, beta and TNF-alpha, which increase the synthesis of PGE2 near preoptic hypothalamus area and thereby triggering the hypothalamus to evaluate the body temperature [10] . So, it will be an advantage to have antimicrobial component in formulation to treat fever. In our studies, extracts of AP, CA, TC, ZO and PN found to have antimicrobial nature. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain, fever, and inflammation. All of the NSAIDs are approximately equivalent in terms of anti-inflammatory efficacy but also cause untoward side effects in a significant fraction of treated patients and this fact frequently limits therapy and also gives scope for research for an alternative drug with less side effects. Many non-steroidal anti-inflammatory drugs have been reported to act either as inhibitors of free radical production or as radical scavengers. Compounds with in rheumatoid arthritis and inflammation and to lead to

potentially effective drugs [11]. AP, PN and TC were found to have strong free radical scavenging activity along with TNF- inhibitory nature.

Conclusion

Among the tested, five extracts from AP, CA, TC, ZO and PN were found to have promising antimicrobial, antioxidant and anti-inflammatory properties. The obtained results might be considered sufficient to further studies to evaluate of possible synergism among extract components and a standardised polyherbal composition based on these active plant extracts may provide us a potent herbal therapy for the treatment of pyrexia.

Table 1: Antimicrobial Effect of Crude Extracts

	MIC Values in µg/ml					
	S.aureous	***E.coli***	***P.auriginosa***	***S.typhi***	***C.albicans***	***S.ceraveciae***
O.corymbosa	>1000	500	50	500	>1000	>1000
T.cordifolia	125	125	250	125	125	125
A.paniculata	125	125	62.5	125	125	125
C.rotundus	500	500	>1000	>1000	>1000	>1000
Z.officianale	125	250	250	250	500	250
P.nigrum	125	125	250	125	125	125
G.pentaphyla	>1000	>1000	500	>1000	>1000	500
E.odoratum	500	500	500	>1000	>1000	>1000
C.aromaticus	250	125	125		125	125

Table 2: *In Vitro* Antioxidant Activity of Plant Extracts

Plant Name	IC_{50} in g/ml*	
	DPPH	**Nitric oxide**
Piper nigrum	180.00 ± 0.38	340.00 ± 0.85
Tinospora cordifolia	96.86 ± 0.80	186.43 ± 1.31
Zingiberis officinallis	51.41 ± 0.51	364.00 ± 0.78
Coleus aroamaticus	45.00 ± 1.01	262.00 ± 0.33
Andrigraphis paniculata	65.00 ± 0.68	182.00 ± 0.81
Oldenlandia corymbosa	120.00 ±0.38	538.00 ±0.68
Cyperus rotundus	181.00 ± 0.44	220.00 ± 0.48
Glycosmis pentaphyla	129.00 ± 0.38	242.00 ± 1.20
Glycosmis pentaphyla	230.00 ± 0.03	430.0 0.08

***** Average of three independent determinations

Table 3: Anti-Inflammatory Properties of Plant Extracts In RAW Cells

Plant name	% TNF inhibition
Piper nigrum	56.50
Tinospora cordifolia	69.15
Zingiberis officinallis	36.73
Coleus aroamaticus	47.56
Andrigraphis paniculata	50.00
Oldenlandia corymbosa	38.75
Cyperus rotundus	21.86
Glycosmis pentaphyla	41.25
Eupatorium odoratum	39.45

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References

1. Simpson BB., Martin MJ., Benjamin B. Economic Botany: Plants in Our World. 1986; 351.
2. Fransworth NR., Akerele O., Bingel AS, Soejarto DD., Guo Z. Medicinal plants in therapy. Bulletin World Health Organization. 1985; 63: 965-981.
3. Joy PP., Thomas J., Mathew S., Skaria BP; Medicinal Plants. 1998; 6.
4. Hatapakki BC., Hukkeri VI. International Journal of Comprehensive Pharmacy. 2010; 2(6): 1-3.
5. Gibbons S., Birgit O., Jhonsen I. Fitoterapia. 2002; 73: 300-304.
6. Jayaprakasha GK., Lingamallu JR., Kunnumpurath KS. Organic Medicinal Chemistry. 2004; 12: 5141-5146.
7. Badami S., Rai RS., Suresh B. J. Ethnopharmacol. 2005; 37(35): 1-5.
8. Varma RS., Godavarthi A., Vidyashankar S., Patki PS., Nandakumar KS. Immunopharmacology and Immunotoxicology, 2011; 33(1): 55-63.
9. Francis D., Rita L. Journal of Immunological Methods. 1986; 89: 271-277.
10. Shah BN., Seth AK. Advances in Bioresearch. 2010; 1(1): 10-16.
11. Asuncio NB., Eleni P., Dimitra HL., Raquel V., Esther V., Beatriz S., Saioa A. Bioorganic & Medicinal Chemistry Letters. 2007; 17: 6439-6443.