ejpmr, 2017,4(8), 643-648

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

A COMPARATIVE STUDY AND PHYTOCHEMICAL SCREENING, DPPH FREE RADICAL SCAVENGING AND IN-VITRO STUDY OF CYTOTOXIC ACTIVITY OF ANDROGRAPHIS PANNICULATE AND LEUCAS ASPERA ON MA104.

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Article Received on 21/06/2017 **Article Revised on 11/07/2017** **Article Accepted on 01/08/2017**

ABSTRACT

Objective: To investigate the phytochemical, cytotoxicity, antioxidant activity of whole Leucas aspera and Andrographis panniculate alcoholic extract on MA104 cells in-vitro using MTT. **Methods:** Andrographis panniculate and Leucas aspera was extracted by absolute ethanol, chloroform, acetone and water. This extract was subjected to phytochemical screening (qualitative), followed by analysis for antioxidant activity (DPPH assay) and ethanol extract was used to investigate the cytotoxic activity of the A.panniculate and L.aspera by in-vitro using 3-(4, 5-dimethyl thiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT). **Results:** Crude ethanolic extract of plant Leucas aspera and chloroform extract of Andrographis panniculate showed to be having antioxidant activity with IC_{50} value of 4.45µg/ml while compared with control ascorbic acid which had an IC_{50} value of 13.5µg/ml. The potency of the plant extract concentration has been calculated using percent decrease in number of viable cells in MA104 compared with control value. **Conclusion:** Based on the crude extract results obtained indicates the presence of phenolic compounds that serves as free radical may be a promising drug candidate that could be used in drug designing for diarrheal and various pharmacologic activities and presence of tumorigenic property indicates it can also be used as candidate to treat cancer after screening of the pure compound in the plant.

KEYWORDS: Phytochemicals, Leucas aspera, Andrographis panniculate, DPPH, MA104, Cytotoxicity.

INTRODUCTION

Human beings utilize plants for basic and preventive and curative health care management since time immemorial. The investigation of medicinal properties of various plants attracted an increasing interest in last two decades because of their potent pharmacological properties, user friendly and low toxicity and due to economic availability.[1] Nevertheless, in several cases, where the host is suffering from prolonged diarrhea and fever, virus-specific treatment will be necessary if possible.[2]

Phytochemicals present in plants, may protect human from a host and lots of diseases. Phytochemicals produced by plants are chemicals that protect plant itself as defense against environmental conditions, but in recent research which demonstrates that this could be used against humans to cure the disease caused by microorganism. The interest to develop and make research in plant derived medicines is basically due to multidrug resistance of antibiotics such as MRSA and recent time widespread of green prescription [3] and development of AYUSH than the synthetic drugs which are costly and possess adverse effects against human systems. The source of most of the active ingredients of medicines and more than 80% of drug substances were natural products or inspired by a natural compound.[4]

Working on the phytochemicals, derives from plants that have different mechanisms based on the source they are extracted such as herbs, barks, stems, fruits, and leaves

or whole plants.[5] Fresh juice of the root is taken to relieve pain associated with kidney. Guava leaves are used internally in dysentery and diarrhoea cases.[6] Root and bark are stimulant and are applied externally for skin eruptions and poisonous bites.[7] As antioxidants have been reported to prevent oxidative stress and damage caused by free radical they can interfere with oxidation process by reacting with free radicals, catalytic metals and chelating agents and also they act as oxygen scavengers.[7,8,9]

Upsurge of interest in reducing plant tissue injury through antioxidants in therapeutic fields has found interest and given scope for scientists to make more and more findings, even though there are several synthetic antioxidants such as ascorbic acid and butylated hydroxyanisole are available in the market but they are unsafe and cause toxicity which is the main drawback.[10] The use of indiscriminate prescription and malpractice of the commercially available drugs has made botanist and scientists for urge of screening of naturally available antimicrobial agents from various sources like medicinal plants which are good sources of novel antimicrobial agents.[11]

Andrographis panniculate a native of India, Taiwan, and Mainland China is an herb with medicinal properties that has bitter taste used to treat liver diseases, colic pain, bowel disorders of children and upper respiratory tract infection and common cold. It grows erect to a height of 30-105 cm in shady, moist places. This plant is locally known as Nilavembu, Siriyanangai and Sirunangai. It is known to have bioactivities such as anti-inflammation, anti-infection, anti-diarrhoeal, anti-hepatotoxicity, anti-diabetes and anti-oxidation.[12]

Leucas aspera belonging to family Lamiaceae, is a common aromatic herb known as Thumbai in Tamilnadu, found throughout in India from Himalayas to Srilanka is known for its various uses in medicinal and agriculture.[13] It is found in dry, open, sandy soil and is abundant in areas where waste materials of household are stacked. Different parts of this plant like leaf, flower, root and stem are found to have antioxidant, antiviral, antibacterial and cytotoxic effect.[14]

The present study is carried out to compare the phytochemical constituents in A.panniculate and L.aspera, and to verify the possible cytotoxic action of A. panniculate and L.aspera on MA104 cells evaluating number of viable cells after incubation with plant extract at different concentrations. This study also investigated the antioxidant effect of whole plants of A.panniculate and L.aspera plant extracts with commercial antioxidant ascorbic acid.

MATERIALS AND METHODS

2.1 collection of plant materials

Based on the documented ethanopharmological knowledge on the use of medicinal plants in the treatment of diarrheal diseases, we choose fresh plants of Andrographis panniculate and Leucas aspera. Plants were collected during the month of July and August from abandoned lands near Kanchipuram. Whole plants and its parts were washed two to three times in distilled water and dried in shadow, and grinded into fine powder using electric grinder. They were labeled separately in brown bottle and stored with proper labeling for further use.

2.2 chemicals and reagents

Absolute ethanol (99.5%), 3-(4, 5-dimethyl thiazol-2yl)-2, 5- diphenyl tetrazolium bromide (MTT), 1,1-diphenyl-2-picrylhydrazyl(DPPH), Ascorbic acid was used as reference standard and positive control for free radical scavenging activity.

2.3 extract preparation

The crude drug was extracted using Chloroform, Acetone, Ethanol and Aqueous. The solution of the extract was filtered through Whatman filter paper no.1 and concentrated using rotary flash evaporator and dried under vacuum. The dried extract was used for further analysis.

2.4 qualitative screening of phytochemicals

The above mentioned extracts were subjected to qualitative screening for the presence and detection of phytochemical groups by established methods.

2.5 antioxidant activity (DPPH assay)

The free radical scavenging effect of plant A and plant B extract and ascorbic acid was assessed with the stable scavenger DPPH by the method described by Oyedemi[15] Briefly the concentrations (5, 10, 20, 50,100,200 µg/ml) were prepared in ethanol. Ascorbic acid was used as reference drug. The solution of 0.135mM DPPH was prepared in ethanol. Different concentration of extract (0.1ml) was mixed with 1.9ml of DPPH solution. The reaction mixture was vortexed thoroughly and left in the dark are room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. Lower absorbance of the mixture indicates the higher scavenging activity of the drug. The ability of plant extract to scavenge DPPH radical was calculated from the following formula:

%DPPH inhibition= [(OD of control - OD of test)/ (OD of control)] x100

2.6 IC50 value of the extract

Inhibition concentration (IC_{50}) value has been determined from the plotted graph of scavenging activity versus the concentration of the extract (using linear scale regression analysis) from the triplicate measurements of the extracts used. The amount of antioxidant necessary to reduce the initial DPPH radical concentration by 50% and lower the IC_{50} value indicates the higher radical scavenging activity.

2.7 Cell Culture

MA 104 an adherent cell line which is used for isolation of rotavirus from faecal samples, responsible for diarrhoea among children. It has origin from African Green Monkey fetal kidney, and has been bought from NCCS Pune. This cell line is used for isolation of human rotavirus from clinical samples.

2.7.1 Preparation of MA104 cell suspension for cytotoxicity activity

MA104 cell was trypsinised, and the cells were suspended in the growth medium containing 10% FCS. The cells were suspended in the medium by gentle passage with the pipette and then cells were homogenized. 1ml of the homogenized cell suspension was added to each well of a 24 well culture plate and incubated at 37°C in a humidified CO2 incubator with 5% CO2. After 48 hrs incubation the cells were observed under an inverted tissue culture microscope. With 80% confluence of cells cytotoxic assay was carried out.

2.7.2 Cytotoxicity Assay

The assay is carried out using (3-(4, 5-dimethyl thiazol-2yl)-2, 5- diphenyl tetrazolium bromide (MTT). MTT is cleaved by mitochondrial Succinate dehydrogenase and reductase of viable cells, yielding a measurable purple product formazan. This formazan production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity.[16,17]

RESULTS

3.1 qualitative screening of phytochemicals

The phytochemical screening showed the presence of alkaloids, flavonoids, trepenoids, tannins, glycosides and phenols. Table 1

3.2 antioxidant activity (DPPH assay)

The free radical scavenging effect of the plant extracts and ascorbic acid was presented in Figure 1. The results showed that the inhibition concentration of chloroform extract of L.aspera exhibited the greatest scavenging activity with a mean percentage of (3.15 µg/ml) at the concentration of µg/ml than chloroform extracts of A.panniculate (35.14µg/ml) whereas the same concentration of ascorbic acid exhibited the mean radical scavenging activity of (21.30µg/ml) [Figure 1a].

3.3 cytotoxicity

The3-(4,5-dimethyl-thiazol-2-yl)-3,5-iphenyltetrazolium bromide (MTT) assay was used to determine the cytotoxicity of A.panniculate and L.aspera crude extracts. MA104 cells were grown (80% of confluency) in 96-well plates for 48 h with growth medium. The media was replaced with without FCS medium containing serial diluted ethanol plant extracts, and the cells were incubated and observed for cytotoxicity after 24, 48 and 72 hours (Figure 2). The culture medium was removed and 20 ml of MTT (Sigma) solution (5 mg/ml in PBS) was added to each well and incubated at 37 °C for 4 h. After removal of the supernatant, DMSO 100ml was added to solubilize the formazan crystals, and the culture was incubated for 30 min. The optical density was measured at 620 nm in an UV ELISA reader. The 50% cytotoxicity concentration (CC_{50}) value was calculated as the concentration that decreased the number of viable cells to 50% of the untreated controls (Figure 3). The selectivity index (SI) was calculated as CC_{50}/EC_{50}.

Table 1: Observation on phytochemical screening of A. panniculate and L.aspera.

Phytochemicals	Chloroform		Acetone		Aqueous		Ethanol	
	A	B	A	B	A	B	A	B
Alkaloids	-	-	+	+	-	-	-	+
Flavonoids	-	-	+	-	-	+	-	+
Phenol	-	-	+	+	+	-	+	-
Quinones	-	+	+	+	+	+	-	+
Tannins	-	-	+	-	-	+	+	+
Carbohydrates	+	+	-	-	-	-	+	-
Steroids	+	+	+	-	-	-	+	-
Glycosides	+	+	+	+	-	-	-	-
Trepenoids	+	-	+	+	+	-	+	+
Ninhydrin	-	-	-	-	-	-	+	-
Saponins	-	-	-	-	-	+	+	-

A= Andrographis panniculate B= Leucas aspera ; +=présence ; -=absence

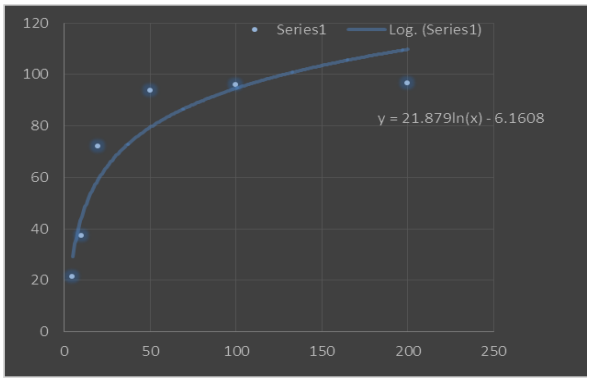


Figure 1: DPPH free radical scavenging activity of Ascorbic acid.

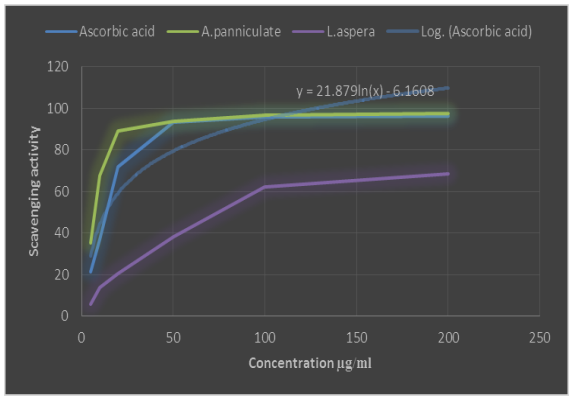
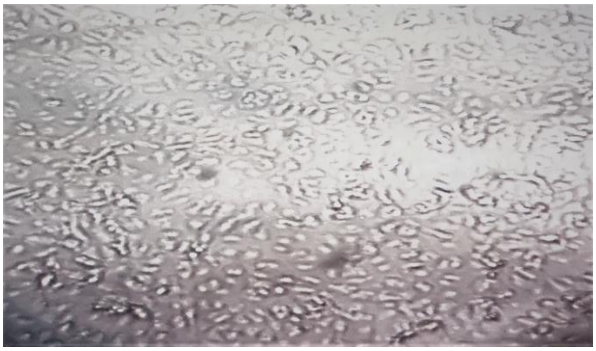


Figure 1: a Free radical scavenging activity of ascorbic acid with L. aspera and A. panniculate.

A



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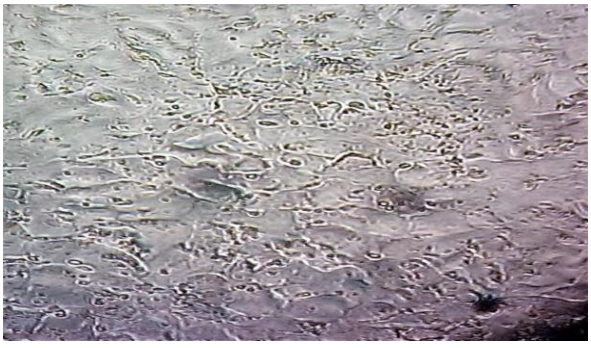

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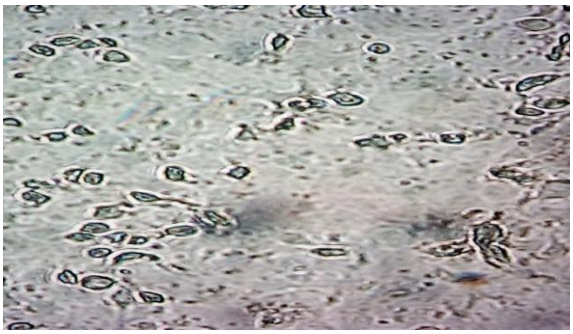

D

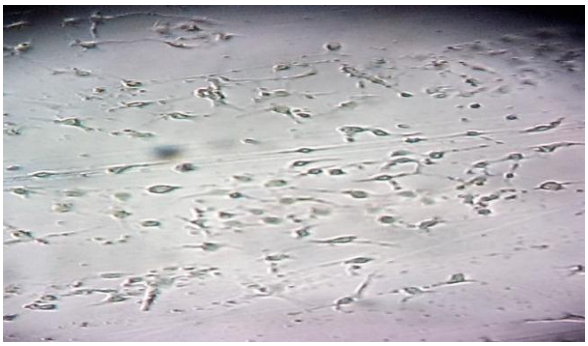

Figure 2: Cytotoxicity of A. panniculate and L. aspera on MA104 cells.
Figure 2.
A) Normal Cell of MA104
B) CPE after 24 hours
C) CPE after 48 hours
D) CPE after 72 hours of incubation

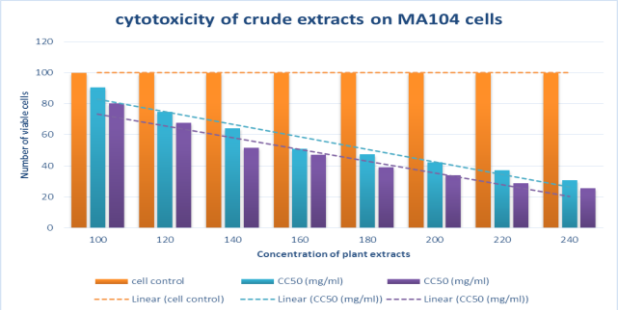

Figure 3: Cytotoxicity of crude extracts of Andrographis panniculate and Leucas aspera.

DISCUSSION

From scientific point of view studies on native or folk plant plays a vital role in finding and development of newer drugs. Plants possess certain chemicals, which have the ability to modify the function of the host cells physiologically and can act as anti-diarrheal, anti-cancer drugs to arrest the proliferation of cells and have been found to possess a wide range of activities which may protect against different chronic diseases. For example, alkaloids protect against chronic disease[18] saponins protect against hypercholesterolemia, triterpenoids show analgesic property, sterols and saponins are also responsible for central nervous system activities.

In the present study it was observed that the plants Andrographis panniculate and Leucas aspera possessed phytochemical and cytotoxic activity by comparison while Andrographis panniculate is found to have more phytochemicals than Leucas aspera in the various extract used in this study. While regarding anti-oxidant activity both plants plays a key role in showing free radical scavenging activity. But both of these plants can be used as drug candidates to cure many chronic diseases.

In a study conducted by Ha-Huyn etal,[19] reports activity of Alpinia katsumadai extracts on MA104 which is used for isolation of rotavirus at 133µg/ml while we observed a concentration of 150µg/ml. Yet another study by Ronner etal., [20]suggest that presence of saponins will 'coat' the epithelium of host small intestine and prevents attachment of virus. Presence of alkaloid, glycoside, sterol, flavonoids, tannin, and trepenoids having anti-diarrheal activity have been reported by Lima et.al.[21] From our study which showed cytotoxic activity may be due to one of the phytochemical compounds tannins presence may play a role against rotavirus in MA104 cells have been documented by Palharse etal.[22]

CONCLUSION

This type of study provides the health application at affordable cost. The experimental plants studied here demonstrates that chloroform extract of whole plant L.aspera has greater promising antioxidant activity than A.panniculate. The cytotoxic effect is principally contributed by the presence of secondary metabolites like alkaloid, glycoside, sterol, flavonoids, tannin, and trepenoids in the extract. This is also consistent with our observation. We observed the ethanol extracts of L.aspera has greater cytotoxicity activity when compared with A.panniculate. It has greater use for human health. Plants in this study possessed scavenging capacity of reducing free radical induced could be source of therapeutic importance. The crude fractions has also has very prominent antitumor activity which can be used in many pharmacological, human therapeutic and various biological actions. However further studies to screen for the bioactive compound present in this plants to treat dreadful diseases has to be conducted to confirm this attribution.

Ethical Disclosures

Protection of human and animal subjects

The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data

The authors declare that no patient data appear in this article.

Right to privacy and informed consent

The authors declare that no patient data appear in this article.

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