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Biological Activities of Plant Ethanolic Extracts from *Asplenium trichomanes* (Maidenhair spleenwort) and *Lagenaria siceraria* (Bottle gourd)

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Abstract

Plant is commonly known as one of the main dietary sources of the human being. Many plant-derived compounds have been used as drugs, either in their original or semi-synthetic form. There are also several plant extracts or "phytomedicine" in clinical trials for the treatment of various diseases. Plant-derived compounds will still be an essential aspect of the therapeutic array of medicines available to the physician, particularly with the availability of new hyphenated primary analytical methods by determining biological activities of medicinal plants. The ethanolic extracts of two creeper plants namely *Asplenium trichomanes* (Maidenhair spleenwort), *Lagenaria siceraria* (Bottle gourd), originated from different geographical part of the world were chosen to be determine its antimicrobial, antioxidants, anti-inflammatory and UV radiation absorbance activity which is mainly characterize the anticancer activity which is nowadays become the most fatal disease. Such kind of biomedical and biotechnology approaches have been aimed to eradicate naturally such illness which is the main objective of this work. The present study shows that different ethanolic leaf extracts have an important and necessary antioxidant and anti-inflammatory properties with UV absorbing activity at UV –A region. These results show that *Asplenium trichomanes* and *Lagenaria siceraria* will be potential natural sources of antioxidant and anti-inflammatory activity. They could have greater importance as therapeutic agents in preventing or treating oxidative stress and inflammation related disorders, such as cancer and other health disorders. While the negative result of the antimicrobial activity of most of both ethanolic extract is considered as a positive result depending to main purpose of future work. Because it shows that extracts will never upset the gastric flora, in which they must be applied safely away from any harmful reaction and allergic or side effects for the living organism.

Keywords: Medicinal plants, ethanolic extracts, antioxidants, anti-inflammatory, antimicrobial, anticancer, Ultra-violet absorbance activities, Asplenium trichomanes (Maidenhair spleenwort), Lagenaria siceraria (Bottle gourd)

1. Introduction

Cancer is a disease with a multifactorial etiology, resulting mainly from genetic alterations, environmental factors and lifestyle (Popim et al., 2008). Up to 10% of invasive cancers are related to radiation exposure, including both ionizing radiation and non-ionizing radiation

(Anand et al., 2008; Balkwill and Coussens, 2004). Exposure to solar ultraviolet (UV) radiation is a causative factor in skin photocarcinogenesis and photoaging. Cancers due to UV irradiation may be a risk and high in future because of the increase in depletion of the ozone layer.

Sun radiation constantly impacts the earth with approximately 50% visible light (400- 800 nm), 40% infrared radiation (IR) (1300- 1700 nm), and 10% ultraviolet radiation (UV) (10-400 nm). UV is divided conventionally to UV-A (320-400 nm), UV-B (290-320 nm), UVC (100-290 nm), and vacuo UV (10-100 nm) (Mensah et al., 2001). Sunscreens are chemicals that provide protection against the adverse effects of solar and in particular UV radiation (Elmets and Young, 1996). Extracts of many plants have been found to possess chemical compounds which act as anti-inflammatory agents and previous research has shown that inflammation is causally linked to carcinogenesis and acts as a driving force in premalignant and malignant transformation of cells (Gomes et al., 2008).

The search for potent natural antioxidants, especially from plant sources, as phytomedicine has become an important research issue at a world-wide level. Many physicians and researchers now contemplate the use of antioxidant treatments as a key strategy for inhibiting or reversing the process of carcinogenesis (Niki, 2010). Traditional medicines play an important role in health services around the globe. About three-quarters of the world population relies on plants and plant extracts for healthcare. The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare. (Singh and Singh, 2008; Prachayasittikul et al., 2008; Shah et al., 2010; Brown et al., 2018; MacDonalds-Wicks et al., 2006; Regoli and Winston, 1999; Stevenson and Hurst, 2007; Halim et al., 2022).

1.1 *Asplenium Trichomanes* (Maidenhair Spleenwort)

Asplenium trichomanes subsp. *trichomanes* is a fern with clusters of 5 to 25 cm long fronds arising from a short, thick rhizome. The small pinnae curl under in dry times though the fern quickly recovers when moisture becomes available. When fertile, ripe sporangia can cover the lower surface of the pinnae (Duncan and Isaac, 1986). The fern can re-sprout from its thick rhizome following fire or other damage. It can be identified year-round, arises from a short, thick rhizome covered with very dark, shiny, coarse, lattice-like scales. Fronds are clustered, erect and 5 to 25 cm long. The stipe is short, brittle, shiny, black and flattened above, with a tuft of scales at its base. The lamina is deep green, linear, pinnate and firm in texture. The rachis is shiny, blackish, brittle and grooved with narrow, fragmented wings. There are 15 to 40 pairs of shortly stalked pinnae arranged asymmetrically. The pinnae are oblong to oval, 4 to 10 mm long with deeply crenate to almost entire margins. The veins are obscure but minor veins branch pinnately from the midvein. After 6 to 12 months, the lower pinnae are often deciduous leaving small projections. Sori on the undersurface of pinnae are arranged in 3 to 6 pairs along the minor veins, oblique to the midvein. Each sorus is protected by a pale, thin indusium with an irregular margin, opening towards the center of the pinna.

1.2 *Lagenaria Siceraria* (Bottle Gourd)

The genus name *Lagenaria* comes from lagena, the Latin name for a Florence flask; referring to the fruit of *Lagenaria siceraria*. The species name *siceraria* probably also refers to the fruit which is useful when it is mature and dry (siccus). Cucurbitaceae family consists of about 120 genera and 735 species. Plants grow mostly tropical and subtropical countries. Many species are cultivated as food plants such as cucumber, melon, pumpkin and watermelon. The plants are perennial herbs and shrubs. The leaves are alternate flowers are mostly unisexual and white or yellow in color; they occur on the same plant (monoecious) or on separate plants (dioecious). The fruit is berry (soft-shelled) or gourd (hard-shelled) with one to many, the seeds are flattened. The genus *Lagenaria* contains six species, probably all originally Old World and

mainly African. Only two species are found in southern Africa; *Lagenaria siceraria* and *L. sphaerica*. (Deshpande et al., 2008)

The increase in prevalence of multiple drug resistance has shown the development of new synthetic antibacterial, anti-oxidative and anti-inflammatory drugs; moreover, the new drug is necessary to search for new antimicrobial, antioxidant and anti-inflammatory sources from alternative sources. Phytochemicals from medicinal plants showing antimicrobial, antioxidant and anti-inflammatory activities have a potential of filling this need because their structures are different from those of the more studied plants (Miguel, 2010). In this growing interest, many of the phytochemical bioactive compounds from medicinal plants have shown many pharmacological activities (Thakur et al., 2013).

2. Materials and Methods

The leaves of *Lagenaria siceraria*, were collected during the month of December, 2013 from The New College Hostel Campus, Royapettah, Chennai, Tamil Nadu, India. Leaves of *Asplenium trichomanes* were collected from Mbeni, Comoros Islands in Africa. Leaves of these plants were brought to Department of Biotechnology, The New College, Chennai, and were washed with sterile distilled water, then shredded into small fragments. The materials were then shade dried at ambient temperature of 32°C for 21 days. The dried samples were then crushed into fine powder using an electronic blender, screened through 1 mm sieve and packed in sterile pouches.

Six experiments were conducted on these samples in order to determine their characteristics. The materials used and the procedures are given below.

2.1 Solvent Extraction

The fine powder of the leaves of *Asplenium trichomanes*, *Lagenaria siceraria* was extracted at 47°C by using soxhlet apparatus using ethanol as solvent (10g in 150ml of ethanol) for 8 hours. After extraction, the dark solution that was obtained was transferred to 250 ml beaker and dried at room temperature for one week by allowing the ethanol to evaporate. After evaporation the extract that was sticked to the beaker was obtained by scraping with the help of a blade and the amount obtained was weighed in a digital weighing balance and stored properly for further studies.

2.2 Phytochemical Analysis

Phyto-chemical analysis was used to determine various phyto-chemicals present in the leaf extracts of *Asplenium trichomanes*, *Lagenaria siceraria*. Test tubes, test tube holders, micropipettes, respective reagents and chemicals, weighing balance, beakers, conical flasks, distilled water and boiling water bath were used in this experiment. Considering alkaloids, to 1ml of extract, diluted hydrochloric acid and Mayer`s reagent was added to form white precipitate. Considering steroids, for Salkowski reaction, to 2ml of extract 2ml of chloroform and 2ml of concentrated sulfuric acid were added. By shaking well, chloroform layer appears red and acid layer shows greenish yellow florescence. Considering coumarins, to 1ml of the extract, 1ml of 10% sodium hydroxide was added to form yellow color. Considering tannins, to 1ml of the extract, 5ml of distilled water and few drops of 1% lead acetate was added to form white precipitate.

Considering saponins, to 1ml of the extract, 5ml of water was added and shaken vigorously to form honey comb like froth. Considering flavonoids (Shinadow's test), to 1ml of the extract, 5ml to 10 drops of diluted HCl and small amount of zinc or magnesium was added and boiled for few minutes to form reddish pink color. Considering anthraquinones (Borntrager's test), 1ml of the extract was macerated with ether and filtered. To the filtrate, aqueous ammonia was

added to form Pink red or violet color after shaking. Considering phenols, to 1ml of the extract, 2ml of distilled water and few drops of 10% aqueous ferric chloride were added to form blue or green color.

2.3 Determination of UV–Absorbance and %T (Transmittance) at UV-A Range

The leaf extracts of *Asplenium trichomanes*, *Lagenaria siceraria* were prepared at a concentration of 2mg/ml in distilled water. The absorbance and transmittance of both leaf extracts were measured at UV–A range (i.e., from 350nm–380nm) using ELICO SL-150 UV-Vis Spectrophotometer, and their absorbance (OD) and transmittance (%T) values were recorded.

2.4 In-Vitro Antioxidant Study of Leaf Extracts

For the determination by reducing power method, test tubes, ferricyanide reagent, ferric chloride reagent, trichloro acetic acid reagent, phosphate buffer solution, centrifuge tubes, centrifuge machine UV-visible spectrophotometer, quartz cuvettes, distilled water, beakers, conical flasks, and test tube holders were used.

In this experiment, the reducing power of different extracts (20 -100 µg/ml) in 1.0ml of deionized water were mixed with phosphate buffer (2.5ml, 0.2M, Ph 6.6) and 1% potassium ferricyanide (2.5ml). The mixture was incubated at 50^{o}C for 20min. Aliquots of trichloroacetic acid (2.5ml, 10%) were added to the mixture, which was then centrifuged at 1036×g for 10min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and a freshly prepared $FeCl_3$ solution (0.5ml, 0.1%).

The absorbance was measured at 700nm and the results of anti-oxidant activity of extract of *Asplenium trichomanes* and *Lagenaria siceraria* using reducing power determination method. In this assay, ascorbic acid was used as control and the Optical Density (OD) values were compared with the sample values.

2.5 In-Vitro Anti-Inflammatory Property

For the Inhibition of albumin denaturation, test tubes, beakers, conical flasks, thermometer, bovine serum albumin reagent, water bath, 1N hydrochloric acid, distilled water, and spectrophotometer were used.

Methods of Mizushima and Kobayashi (1968) and Sakat et al. (2010) were followed with minor modifications. The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37^{0}C HCl. The sample extracts were incubated at 37^{0}C for 20 min and then heated to 51^{0}C for 20 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660nm. The experiment was performed in triplicate to obtain the percent inhibition of albumin denaturation. In this assay, aspirin was used as control at a concentration of 100µg/ml and the OD value was compared with the leaf extract samples.

Percent inhibition of protein denaturation was calculated as in Eq. (1):

$$\% \text{ inhibition} = [\{\text{Abs control} - \text{Abs sample}\}/\text{Abs control}] \times 100 \quad (1)$$

where, *Abs control* is the absorbance without sample and *Abs sample* is the absorbance of the sample extract/standard.

2.6 Antimicrobial Activity

Table 1 displays the media composition antimicrobial activity experiment. The materials used were, Mueller-Hinton agar plates, antibiotic discs forceps, 18- to 24-hour old pure culture of the organism to be tested, vortex, sterile swabs, inoculating loop, and Bunsen burner.

Table 1. Media composition for Mueller-Hinton agar per liter of distilled water

Beef, infusion from	300.0 g
Casamino acid, technical	17.5 g
Starch	1.5 g
Agar	17.0 g

Antimicrobial discs were purchased from a reputable supplier, HIMEDIA. Sealed cartridges containing commercially prepared paper discs were stored at either 8°C or frozen at -14°C in a non-self-defrosting freezer. Semiautomatic disc dispensers were used to place the disc on Muller Hinton Agar. A 0.5 McFarland standard was prepared.

The steps of the procedure were (1) Preparation of Mueller-Hinton plate, (2) Preparation of inoculum, (3) Inoculum application, (4) Inoculation of the Mueller-Hinton plate, (5) Placement of the antibiotic discs, (6) Displacement, (7) Incubation of the plates (at a temperature range of 35°C ± 2°C), (8) Measuring zone sizes.

3. Results

3.1 Solvent Extraction

Ethanolic extracts of *Asplenium trichomanes* and *Lagenaria siceraria*, were obtained by soxhlet extraction and they were weighed and dissolved to prepare stock solutions of various concentrations according to Table 2.

Table 2. The concentrations of the stock solutions

Asplenium trichomanes	1gm/ml
Lagenaria siceraria	1gm/ml

3.2 Phytochemical Analysis

Phytochemical analysis was carried out for the determination of various chemical compounds and the results were depicted in Table 3.

Table 3. Results of the phytochemical analysis

S. No	Compound	*Asplenium trichomanes*	*Lagenaria siceraria*
1	Alkaloids	Present	Present
2	Steroids	Present	Present
3	Coumarins	Present	Present
4	Saponins	None	Present
5	Phenolics	Present	Present
6	Flavonoids	Present	Present
7	Tannins	None	None
8	Anthroquinones	None	None

3.3 Determination of UV–Absorbance and %T (Transmittance) at UV-A Range

The UV light absorption and transmittance of both extracts was recorded at UV-A range from 350nm to 380nm and the results were recorded and depicted in Table 4.

Table 4. UV–absorbance and %T (Transmittance) at UV-A range

S. No	UV Range (nm)	*Asplenium trichomanes*		*Lagenaria siceraria*	
		OD	%T	OD	%T
1	350	0.780	16.6	1.746	2.0
2	360	0.584	26.1	1.154	7.0
3	370	0.450	36.4	0.714	19.4
4	380	0.364	43.2	0.461	34.6

3.4 In-Vitro Antioxidant Study of Leaf Extracts

The absorbance was measured at 700 nm and the results of anti-oxidant activity of extract of *Asplenium trichomanes* and *Lagenaria siceraria* were determined from the ferric reducing activity. The results are compared in Figure 1.

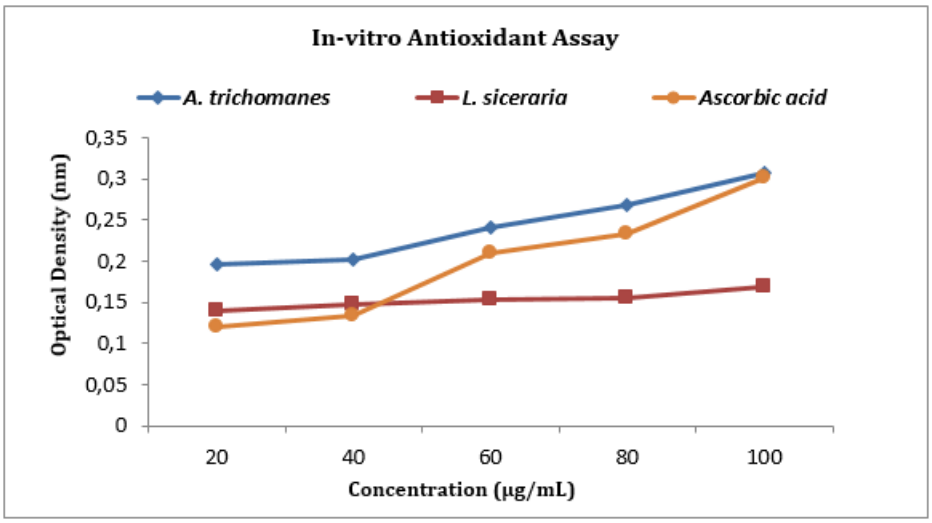

Figure 1. Anti-oxidant activity determination by reducing power method

3.5 In-Vitro Anti-Inflammatory Property

The percent inhibition of albumin denaturation was determined by reading the color (Figure 2) at 660nm and the absorbance values were recorded. The percent inhibition values of the samples were depicted in Table 5.

Table 5. Optical Density for in-vitro anti-inflammatory assay

S. No	Concentration (in µg/ml)	*Asplenium trichomanes*	*Lagenaria siceraria*
1	100	0.324 ± 0.00058	0.297 ± 0.00058
2	200	0.280 ± 0.00058	0.234 ± 0.00058
3	300	0.264 ± 0.00058	0.224 ± 0.00058
4	400	0.244 ± 0.00058	0.203 ± 0.00058
5	500	0.179 ± 0.00058	0.183 ± 0.00058
Control		0.401	
Aspirin (100µg/ml)		0.120	

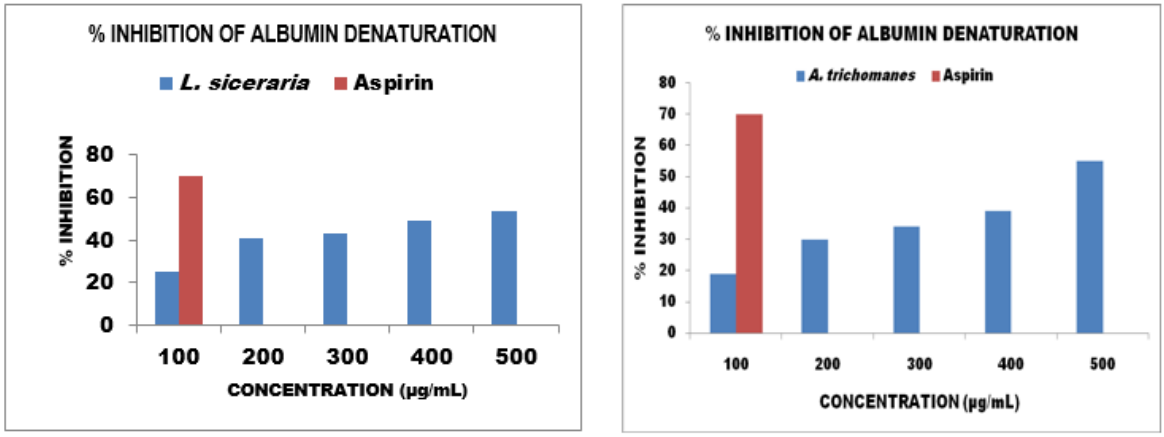

Figure 2. Percent inhibition of albumin denaturation of the extracts

3.6 Antimicrobial Activity

The antimicrobial activity of both plant extracts against *Escherchia coli, Salmonella typhi*, and *Pseudomonas aeruginosa* were recorded from the formation of zones size. It has been found that the antimicrobial activity of the plant extracts shows minimum zone of inhibition.

4. Discussion

Increase in reports about cancer is a serious challenge to the health of the population of the world, and particularly the rate of skin carcinoma is found to be high and the impacts are worsening day by day. The UV radiations are found to be an important factor in causing skin carcinoma and leukemia. Epidemiology and experimental studies have implicated oxidative cellular damage arising from an imbalance between free radical generating and scavenging systems as the primary cause of cancer and various diseases.

The extraction method for the two creepers was applied by the soxhlet apparatus with ethanol as solvent only. This leads to determination of polar compound, further studies are supposed to determine all the compounds by using different type of solvents. The qualitative determination of potent phytochemicals leads to prove the antioxidant activity of plants, it is the case of phenols, flavonoids, tannins, saponins etc…

The antioxidant activity shown by *Asplenium trichomanes* which has been originated from Comoros Islands. A moderate anti-inflammatory activity was specifically determined in the ethanolic *Lagenaria siceraria* which is originated from Chennai-India. This quantitative variation can be explained by the growth media and conditional climate of the plants which provide generally the sufficient nutrients available and sufficient for such kind of characteristics. Most of these plant extracts possess an anti-inflammatory activity which is approximately more than 50%.

Albumin protein denaturation know as coagulation is defined as the process in which proteins lose their tertiary structure and secondary structure by application of external stress such as heat and strong acid or base. The external stress used in this work was the heat which normally leads to the coagulation of protein. This change of characteristic is considered as the inflammation condition.

Owing to that, the addition of the plant extract which react with protein to inhibit coagulation of Albumin which will express a high transparence of the light while using the spectrophotometer. This high transparence defines a low absorbance of the light which will be less than the control absorbance and give a good result while applying the mathematic formula of the inhibition. It was effective in inhibiting heat induced albumin denaturation.

Inhibition of 54%, 55% were respectively observed at 500µg of *Lagenaria siceraria* and *Asplenium trichomanes* extracted in 1ml of ethanol. Aspirin as a standard anti-inflammation drug showed the maximum inhibition 70% at the concentration of 100µg/ml compared with control. The negative result of the antimicrobial activity of both ethanolic extract is considered as a positive result depending to main purpose of future work. Because it shows that both of these extracts will never upset the gastric flora, in which they must be applied safely away from any harmful reaction and allergic or side effects for the living organism.

The combination of these different medicinal plants is expected to produce a high and effective reaction against cancer and other cardio-vascular diseases. Above discussion support that there may be the presence of different polyphenolic compounds such as flavonoids, tannins, terpenoids, phenols, Saponins which are necessary components characterizing the antioxidant and anti-inflammatory properties of drugs.

5. Conclusion

The present study shows that different ethanolic leaf extracts have important and necessary antioxidant and anti-inflammatory properties with UV absorbing activity at UV –A region. These results showed that *Asplenium trichomanes* and *Lagenaria siceraria* could be potential natural sources of antioxidant and anti-inflammatory activity. They could have greater importance as therapeutic agents in preventing or treating oxidative stress and inflammation related disorders, such as cancer and other health disorders. Further studies are planned to be achieved by determining the UV stability of these plants, and to assess the in- vivo biological activities. This will lead to determine the active component characterizing these different biological activities.

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Author Statement

The authors confirm contribution to the paper as follows: study conception and design: S. N. Rahaman, M. A. I. Musthafa; data collection: M. C. Mkouboi; analysis and interpretation of results: All Authors; draft manuscript preparation: M. C. Mkouboi. All authors reviewed the results and approved the final version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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