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AN IN VITRO ASSESSMENT OF THE PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITIES OF ACHYRANTHES ASPERA LEAF AND MESUA FERREA FLOWER EXTRACTS

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Abstract:

This study aimed to look for phytochemicals in 60 % methanolic, 60 % ethanolic and aqueous extracts of Achyranthes aspera leaves and Mesua ferrea flowers and evaluate their antioxidant potential using free radical scavenging activity (DPPH). The dried powders of the botanicals were also subjected to the study. The flavonoids, phenols, tannins and carbohydrates were identified in the methanol and aqueous extracts of A. aspera and also in methanol and ethanol extracts of M. ferrea. The saponins were only present in the dried powder and ethanol extract of A. aspera. The presence of alkaloids was only seen in the dried powders. The mean IC_{50} DPPH values for the dried powders and the extracts of ethanol, methanol and aqueous extracts of A aspera were found to be 65.67, 58.33, 21.20 and 36.41 µg/ml, respectively. While comparing with the DPPH activity on the dried flowers of M. ferrea it exhibited significantly lower activity than the A. aspera. The findings of this experiment suggest that A. aspera has much more antioxidant activity than M. ferrea, and has greater therapeutic properties for humans that can be utilized by the food industry in substituting it with synthetic antioxidants.

Keywords: Achyranthes aspera, Antioxidant, Mesua ferrea, Phytochemicals, plant extracts.

Introduction

The supplementary or conventional medical system, in conjunction with a variety of socio-religious traditions, has a significant impact on healthcare in the Indian subcontinent. The WHO reports that more than two-thirds of the world population relies on traditional herbal remedies to heal their ailments [1]. India produces a high volume of quality medicinal plants and is the world's second-largest exporter. It is considered one of the world's 12 major biodiversity hotspots, with 16 agro-climatic regions and rich diversity of approximately 45,000 plant species, of which 7000 are medicinally used [2]. Global trade in herbal medicines and their derivatives is worth about $100 billion right now, and it's growing at a rate of about 15% a year[3].

Oxidative rancidity is a major issue occurring in foods with high-fat content. It not only degrades the food quality but also contributes to the development of certain disorders such as carcinogenesis, mutagenesis, aging and arteriosclerosis [4]. Numerous naturally occurring antioxidant substances derived from plants have been reported to

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scavenge the free radicals [5]. Recently, there has been an increased interest in identifying naturally existing antioxidants. Their application in foods and therapeutic materials has increased dramatically after the ban on some potent synthetic antioxidants that leads to cancer. Natural antioxidants can safeguard the human body from free radicals, decrease the development of various chronic disorders and inhibit the oxidative rancidity of lipids in food [6]. Many plant phenolics are said to have antioxidant capabilities that are far superior to vitamins E and C [7]. As a result, plants with high polyphenols are extremely important as natural antioxidants. Furthermore, the structural complexity of these plant-derived compounds has contributed significantly to their broad therapeutic effects [8]. This has demanded a more radical approach in the hunt for new antioxidant and antimicrobial compounds from diverse sources.

Hydranths asp era L. is a weedy annual plant native to India. This traditional herbal medicinal plant is widely utilized by ethnic communities to treat various ailments. The entire plant and its components have been extensively examined for their pharmacological actions, and it has established itself as a versatile plant with a broad range of therapeutic effects [9]. The plant has several medicinal and pharmacological properties, including spermicidal, anti-allergic, cardiovascular, nephroprotective, and abortifacient, and antiphrastic, hypoglycaemic, analgesic and antipyretic activity. In addition, it's being used to cure several illnesses, namely boils, bronchitis, colds, cough, colic, debility, dropsy, dysentery, ear problems and headaches [10].

Mesua ferrea is an evergreen, large, glabrous tree. Flowers are white and fragrant, 2 to 10 cm across, with axillaries found in single or pairs. M.ferrea is used in many conventional treatments for various disorders, including rheumatism, piles, asthma, ulcers, helminthiasis, dysentery, bleeding and inflammation [11]. According to published research, the plant has major biological qualities such as antibacterial, anticancer, antiseptic, antioxidant, antiasthmatic and immunomodulatory capabilities [12]. The focus of this research was to examine the chemical components and antioxidant properties of the dried powder and the extracts made from Achyranthes aspera leaves and Mesua ferrea flowers so that they can be incorporated into the food system as a replacement to synthetic antioxidants.

2. Material and Methods

2.1 Plant material:

The leaves of the A. aspera were picked from the campus of the Centre for Medicinal Plants Research Institute, Kottakkal, Arya Vaidya Sala, Kerala. The dried flowers of M. ferrea were collected from the Indian Institute of Spices Research, Calicut, Kerala. The specimens were authenticated and identified taxonomically.

2.2 Preparation of Plant Extract:

To eliminate dust and other exterior pollutants, fresh A. aspera leaves were cleaned with distilled water. The leaves were then dried in shade with appropriate ventilation

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at room temperature and later it was dried in hot air oven at 40°C for 24 hours. Using a mechanical grinder, the thoroughly dried leaves were ground, and the obtained powder was packed in airtight bottles. Similarly, the dried flowers of M. ferrea were also ground, and the powder was stored in airtight bottles.

The dried powder of measured quantity (50g) were soaked in 500ml of different solvents like 60% methanol, 60% ethanol and water. The mixture was kept for 48 hours to obtain the extract. The crude extracts obtained were brought down to 50 ml by rotary vacuum evaporator and then placed in a refrigerator for further study. The dried powders of A. aspera and M. ferrea were also taken into account, along with the extracts. The analysis was carried out in triplicates.

2.3. Preliminary phytochemical analysis:

Sample such as dried powder, ethanol, methanol and aqueous extract were quantitatively estimated for their bioactive components such as alkaloids, carbohydrates, flavonoids, phenol, saponins and tannins.

Test for Alkaloids (Dragendorff's test): Along the sides of the tube, 1 ml of Dragendorff's reagent was added to 2 ml of the crude extracts. The occurrence of alkaloids was indicated by the development of an orange or reddish-brown precipitate [13].

Test for Carbohydrates (Benedict's test): The extract, on boiling with 2ml of Benedict's reagent, developed a reddish-brown precipitate, indicating carbohydrates' presence [14].

Test for Saponins (Foam test): The crude extract was mixed with distilled water (5 ml) and shaken briskly in a test tube. The presence of saponins was determined by the production of stable foam [13].

Test for Flavonoids (Shinoda test): The crude extract was combined with a few shredded pieces of magnesium ribbon. Conc. HCl was added dropwise. A few minutes later, the development of a pink-scarlet color confirmed the existence of flavonoids [13].

Test for Tannins (Ferric chloride test): To 2 ml of an extract with 2 ml of distilled water, a few drops of $FeCl_3$ were added. The development of a green precipitate indicates the existence of tannins [15].

Test for Phenols- (Libermann's Test): Extracts were combined with a few sodium nitrate crystals and gently heated. 1ml of conc sulphuric acid was added after cooling. A red-coloured compound will be formed if a phenol group is present and will turn into deep blue color with the addition of sodium hydroxide [16].

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Antioxidant activity by DPPH radical scavenging activity

The antioxidant activity was measured using the stable 2,2-diphenyl-1-picrylhydrazyl DPPH radical [17]. When the antioxidant compounds in the extracts combine with DPPH, they form di-phenyl hydrazine, a yellow compound. (1.5ml DPPH+1ml methanol+0.5ml sample extract were vortexed for 5 minutes before incubating at 37^0C for 30 minutes.). The degree of discoloration (the reducing ability of antioxidants towards DPPH) from purple to yellow was measured at 516 nm by a UV spectrophotometer, which measures the scavenging potential of plant extracts. The degree to which the purple-colored DPPH solution decolorizes shows its free radical scavenging activity. The antioxidant ascorbic acid was used as a positive control. DPPH in equivalent solvents (without plant material) aids as a blank. The plant extract's DPPH radical scavenging activity was evaluated as percentage inhibition.

$$\text{Inhibition} = \left[\frac{(\text{Control Absorbance} - \text{Sample Absorbance})}{\text{Control Absorbance}}\right] \times 100$$

3. Results and Discussion:

3.1 Phytochemical constituents in A.aspera and M. ferrea

Phytochemical analysis of the methanol, ethanol and aqueous extracts of A. aspera confirmed the existence of important metabolites (Table 1). The methanolic and aqueous extracts had the presence of flavonoids, phenols, tannins, and carbohydrates. While the ethanolic extracts exhibited the presence of saponins along with other phytoconstituents. The dried powder of A. aspera showed the presence of alkaloids which were not evident in the extracts. This might be explained by the soluble nature of phytochemicals in a range of solvents with different polarities. Owing to these phytochemicals in the plant, A. aspera may be used as an antioxidant source.

The phytochemical analysis of the flowers of M. ferrea indicated that the methanol and ethanol extracts had the presence of carbohydrates, tannins, phenols and flavonoids. In contrast, the water extract did not show the presence of flavonoids. This must be due to the resistance of the phytochemical to get dissolved in water. A study on the methanol and ethanol extracts of M. ferrea fruit contained saponins [12]. However, the presence of saponins were not found in this study. The dried powder from the flower displayed the existence of tannins, phenols, carbohydrates, alkaloids and flavonoids. The presence of flavonoids and phenols are considered to be very important compounds medically [9]. Several studies reported that phenolic compounds have been useful in preparing antioxidant compounds [8].

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Table 1: Phytochemical profile of leaves of A.aspera and flower of M.ferrea extracts and powder

	Alkaloids	Flavonoids	Phenols	Saponins	Tannins	Carbohydrates
AA Powder	+	+	+	+	+	+
AA Methanol	-	+	+	-	+	+
AA Ethanol	-	+	+	+	+	+
AA Water	-	+	+	-	+	+
MF Powder	+	+	+	-	+	+
MF Methanol	-	+	+	-	+	+
MF Ethanol	-	+	+	-	+	+
MF Water	-	-	+	-	+	+

AA: A. aspera, MF M. ferrea, +: Present, -: Not present

3.2 Antioxidant activity

The ethanolic (60%), methanolic (60%) and water extracts of A. aspera leaves were shown to exhibit a scavenging action on DPPH radicals and the findings were reported in Fig 1. The mean IC_{50} values for DPPH radical for the crude extracts of ethanol, methanol and aqueous were 58.33 µg/ml, 21.20 µg/ml and 36.41 µg/ml, respectively. The dried powder of the A aspera leaves showed an activity of 65.67 µg/ml. The mean IC_{50} value of reference antioxidant ascorbic acid was 28.12 µg/ml. The data indicate that the ethanolic extracts exhibited good antioxidant activity, followed by the aqueous extract, and the least activity was observed in the methanolic. It was also observed that the extracts had greater free radical scavenging activity than the reference used. The better free radical scavenging abilities of ethanolic extract and the aqueous leaf extract may be associated with their flavonoid and phenolic components [16]. This implies that the plant extracts include compounds that are able enough to donate hydrogen free radicals so that the odd electron responsible for the reactivity of radicals will be eliminated [18]. The difference in antiradical activity observed between the dried powder (65.67 µg/ml) and the extracts might be because of the additional phytochemicals such as alkaloids and saponins. The present study's findings agree with recent research on a large variety of herbal plants.[3]. Similar results has been also been reported that the methanolic extracts exhibited superior antioxidant activity than the petroleum extracts [19],

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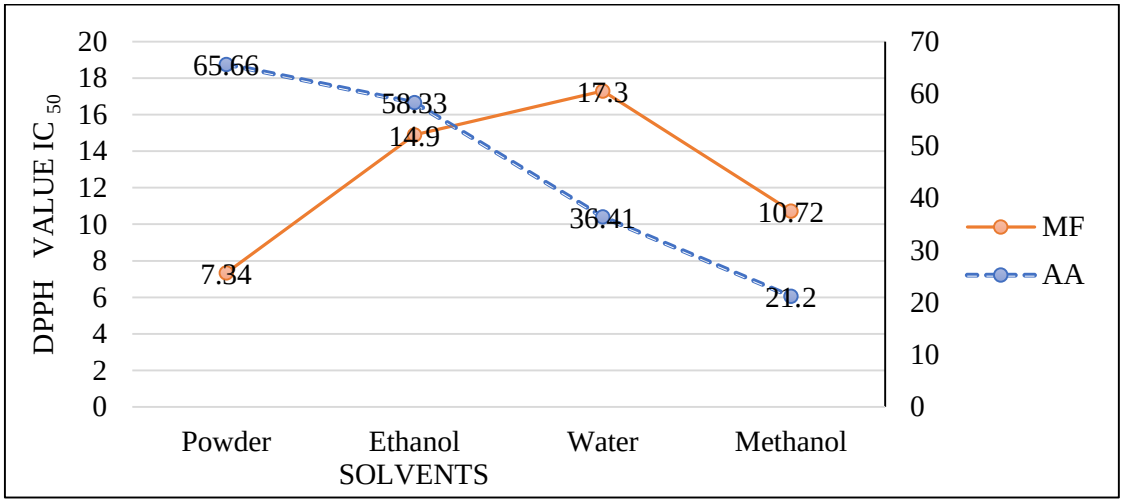

Figure 1: Graphical representation of DPPH activity

While comparing with the DPPH activity on the dried flowers of M. ferrea exhibited a significantly low activity than the A. aspera. The mean IC_{50} values of ethanolic, methanolic and aqueous extract of M. ferrea were 14.92 µg/ml,10.72 µg/ml and 17.2 µg/ml, respectively (Table 2). A study on the extract from leaves of M.ferrea exhibited a similar pattern of DPPH activity. According to the findings, the water extracts were more efficient DPPH radical scavengers than the alcoholic extracts [12]. While another study on the M.ferrea roots reported that that the polar extract (methanol) was more efficient over less polar and non-polar extracts[20]. However, the research on the DPPH scavenging experiment on M. ferrea flowers with hot water exhibited a potential antioxidant activity. Water extracts of M. ferrea flowers had more antioxidant activity than the reference butylated hydroxytoluene, with IC_{50} value of 7.49 and 6.95 µg/ml, respectively [21], which correlates with the present study. The activity on the stem bark of M. ferrea conveyed that in vitro antioxidant activity of chloroform and methanol extracts had good antioxidant activity [22]. The n-hexane extract of M. ferrea stamens possess good free radical scavenging activity with an IC_{50} value of 66.3 µg/ml [23]. However the maturity stage, blooming time, seasonal fluctuations and distinct species can all impact the phenolic biosynthesis [24].

Table 2: Antioxidant activity of extracts and dried powder from A.aspera leaves and M.ferrea flower

	DPPH IC_{50} µg/ml
Ascorbic acid	28.12 ± 1.01
AA Powder	65.66 ± 2.44
AA Methanol	21.20 ± 1.49
AA Ethanol	58.33 ± 1.67

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AA Water	36.41 ± 2.22
MF Powder	7.43 ± 1.16
MF Methanol	10.72 ± 0.55
MF Ethanol	14.92 ± 1.65
MF Water	17.3 ± 1.01
AA: A. aspera, MF M. ferrea : *Results are presented as mean ± standard derivation	

Conclusion and future aspects.

The research on the extracts and the dried powder obtained from Achyranthes aspera leaf and Mesua ferrea flowers has shown that they are rich in phytochemicals. The assessment of antioxidant activities showed promising results. These findings suggest that A. aspera is far more active than M. ferrea thus, there lies an enormous scope for utilizing the properties of these herbs into various kinds of food formulations. Indeed, both antioxidant activity and phenolic content must be examined when assessing the antioxidant potential of plant extracts. Correlation and clustering statistical approaches using antioxidant data and phenolic compound screening might reveal which chemical combinations impact additional activities.

Conflict of Interest: "No conflict of interest associated with this work".

Contribution of Authors: We, Anusha R, Dr. Jyoti Prabha Bishnoi and Dr. Saleem Siddiqui declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors".

The data collection its analysis and the writing of the manuscript was performed by Anusha R, while the final judging and evaluation was performed by Dr. Saleem Siddiqui and Dr. Jyoti Prabha Bishnoi.

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