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STUDY OF PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES OF *MADHUCA INDICA* AND *ACACIA FARNESIANA* SEED OILS FROM MEDICINAL PLANTS OF RAJASTHAN

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ABSTRACT

Introduction: Rajasthan, a region with a diverse flora, has provided the ideal environment for the growth of the medicinal plants. Medicinal plants have played a crucial role in traditional healthcare system; *Madhuca indica* and *Acacia farnesiana* are two such plants that have been integral to the folk medicine of Rajasthan, India.

Objectives: This study aims to investigate the physicochemical and antioxidant properties of these two plant seed oils, shedding light on their potential therapeutic applications. **Methodology:** The seed oils were extracted by using soxhlet extraction method employing n-hexane as solvent. The evaluation of antioxidant activity was carried out through DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay.

Result: The Iodine value in both the seed oils was found to be relatively low, indicating their oxidative stability. Both MISO and AFSO have notable antioxidant activities demonstrated by their low IC_{50} values. The IC_{50} values for the MISO and AFSO were 4.54± 1.15 ug/mL and 7.24± 1.19 ug/mL respectively.

Conclusion: *Madhuca indica* and *Acacia farnesiana*, two prominent medicinal plants of Rajasthan, their seed oils exhibit promising physicochemical characteristics and potent antioxidant properties. These findings provide a strong scientific basis for the traditional use of these plants seed oils in traditional medicine systems and may lead to the development of novel herbal formulations for a range of health conditions.

Keywords: *Madhuca indica*, *Acacia farnesiana*, physicochemical characteristics and antioxidant properties

INTRODUCTION:

Rajasthan is a northern Indian state renowned for its abundant medicinal plant diversity and rich biodiversity. These plants, which are recognized for their rich biodiversity and variety of medicinal uses, have been utilized in Ayurveda for a long time. These plants have long been used in both local folk medicine and Ayurveda, the traditional Indian medical system. The current study focuses on analyzing the physicochemical and antioxidant characteristics of two Rajasthan medicinal plants, *Accacia farnesiana* seed oil (AFSO) and *Madhuca indica* seed oil (MISO). Mahua, also known as *Madhuca indica*, is a valuable and adaptable medicinal plant that grows throughout Rajasthan and other parts of India. This plant has a long history of traditional uses and is a member of the sapotaceae family. Its flowers are used to induce the production of alcohol when making asavas and arishtas, two popular ayurvedic formulations **[1]**. *M. indica* is a large, shade-loving deciduous tree that grows both wild and cultivated throughout much of central India. Its tree is broad uses for its timber, fruits, seeds, and flowers make it significant economically **[2]**. Mahua has a number of pharmacological properties and the potential to improve human health. Anti-diabetic, anti-ulcer, hepatoprotective, anti-pyretic, anti-fertility, analgesic, anti-oxidant, emetic, dermatological, laxative, tonic, anti-burn, anti-earth worm, headache from wound healing, and many other issues are among its many uses **[3]**. The primary pathological cause of many diseases is oxidative stress. Oxidative stress induced by free radicals causes damage that includes aging, cancer, rheumatoid arthritis, tissue injury, and neurodegenerative diseases. Antioxidants are chemicals that prevent target molecules from oxidative deteriorating. The food industry has been devoting time and resources to search for the natural antioxidants in order to find alternatives to synthetic compounds. Numerous investigations have been conducted to assess the antioxidant activity of various plant extracts. Bark of the *M. indica*

species has been shown to have free radical scavenging activity, leprosy patients can be treated with an astringent and emollient made from the bark extract for wounds, swelling, itching, and fractures **[4]**. Madhuca plant extract's gas chromatography-mass spectrometry profile demonstrated the presence of prospective antioxidants **[5]**. *A. farnesiana*, also referred to as Vachellia farnesiana or Sweet Acacia, is a plant that is used medicinally and grows in Rajasthan and other places. This adaptable member of the fabaceae family is valued for both its ecological significance and its traditional medical applications. The sweet acacia tree grows to a height of 4.6 to 7.6 meters, making it a small to medium-sized tree or shrub. The foliage appears feathery, and because the trunk and stems bend easily, the canopy forms an umbrella. The leaves are alternately arranged, bi-pinnately compound, and even. Long, dark brown leguminous pods are the fruits **[6]**. The activity of tested extracts and isolated compounds against tuberculosis and dysentery bacteria supports the ethnomedical use of *A. farnesiana* fruits to treat these diseases **[7]**. *A. farnesiana* has been used to treat diarrhea, headaches, and tuberculosis **[8]**. The antioxidant protection of the extracts from *A. farnesiana* and A. shaffneri pods reported the potential transmission of antioxidant components and protective effects to animal-derived products (milk, meat, and by-products) when this crop is included in their diet **[9]**. Antioxidant, antidiarrheal, and analgesic properties were found in the methanollic extract of *A. farnesiana* , which suggests that the plant contains physiologically significant phytoconstituents **[10]**. The images of *M.indica* and *A. farnesiana* plant with seeds are given in **Figures 1 and 2**.

Thus, the objectives of the current investigation is to examine the physicochemical characteristics and assess antioxidant potential of MISO and AFSO using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, which are grown in semi-arid zone of Rajasthan. The graphical abstract for the present research work is given in the **Figure 3**.

Figure 1: Plant with seeds *M.indica*

Figure 2: Plant with seeds *A. fernasiana*

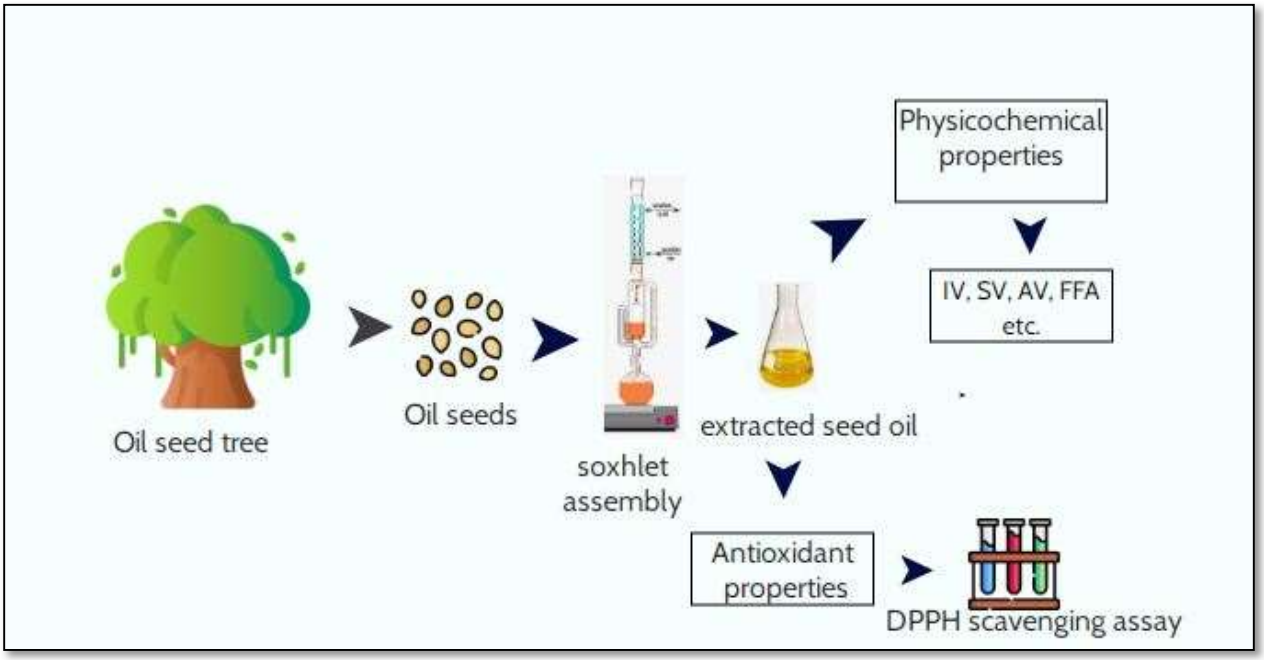


Figure 3: Graphical abstract for the physicochemical and antioxidant properties analysis of MISO and AFSO

MATERIALS AND PROCEDURES:

Seed collection and sample preparation: *A. farnesiana* and *M. indica* seeds were collected from several Rajasthan districts. After being washed with water, the seeds were shade-dried, and then they were crushed and ground using a mortar.

The following chemicals and standards were purchased from Sigma Aldrich Chemical: n-hexane, potassium hydroxide, sodium hydroxide, sodium thiosulphate, acetic acid, chloroform, diethylether, Wij's solution, DPPH, and ascorbic acid. Every chemical and solvent used was of the analytical grade.

Oil extraction: A soxhlet apparatus was utilized to extract the MISO and AFSO with n-hexane (40^0–60^0C) serving as the solvent. For six hours, the extraction was done at the solvent's boiling point. Following the extraction, the solvent and oil were separated by evaporating them at 40°C in a rotary vacuum evaporator.

Examination of Physicochemical Characteristics: The MISO and AFSO were examined for their physicochemical properties such as density, moisture content, and refractive index (RI). The RI was measured by Abbe's refractometer. Using AOCS standard procedures, the saponifaication value (SV), acid value (AV), and Free fatty acid (FFA) content were calculated **[11]**. Wij's method was used to determine the iodine value (IV). The iodometric titration method serves as the foundation for this, wherein the unsaturation compound reacts with iodine that is indirectly produced **[12, 13]**. The protein content was determined using kjeldahl method **[14]**.

Examining the activity of antioxidants:

Using DPPH free radical scavenging activity, MISO and AFSO antioxidant properties were assessed. The method for calculating the DPPH free radical scavenging activity was developed by Cheung LM *et al* **[15]**. To describe it briefly, 0.2 mM DPPH reagent and methanol was added to each extract at a 4:1 volume ratio. Using a spectrophotometer, the absorbance was measured at 520 nm following ten minutes of exposure to low light. The standard used is ascorbic acid. The antioxidant activity of MISO and AFSO was measured by the formula given below:

$$\text{DPPH free radical scavenging activity} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100$$

The data was displayed as IC_{50} (inhibitor concentration) values, which are the sample concentrations that cause a 50% reduction in the DPPH reagent's absorbance.

RESULT AND DISCUSSION:

Physicochemical properties of MISO and AFSO:

Table 1 displays the physicochemical characteristics of MISO and AFSO that have been studied. The seed oils of MISO and AFSO were greenish-yellow and golden yellow colour respectively. The odor from AFSO and MISO was sweet and very faint, respectively. The oil content of *M. indica* and *A. farnesiana* seeds was discovered to be 48% and 25%, respectively. These results were found to be comparable to the oil content obtained by Mekala NK and Khan U.I for these seed oils **[16, 17]**. The moisture contents of the seeds of *M. indica* and *A. farnesiana* were found to be 0.25% and 0.6%, respectively. A number of physicochemical properties, such as the moisture content and SV of edible seed oils, are significant in determining their quality and, consequently, their economic value. The relative degree of unsaturation in oils is expressed as the Iodine Value (IV). The unsaturation and oxidation susceptibilities increase with increasing iodine values. Because of its lower IV=6, MISO may have had greater oxidative storage stability. The IV obtained for MISO is significantly less than the IV=70 as reported by Dugala NS **[18]**. The AFSO has IV=110 which is significantly lower than that of A. Arabica (IV=144.50) as experimented by Al Juhaimi, F **[19]**. The degree of conjugation, degree of unsaturation, fatty acid chain length, and molecular weight are all related to the RI **[20]**. The MISO and the AFSO yielded RI of 1.462 and 1.467, respectively. These results are within the range (1.454-1.4691) for various edible oils that Mengistie *et al*. reported **[21]**. The SV is used to detect adulteration. The observed SV for MISO was 198.50 mg $KOH.g^{-1}$ which fell within the range (198.3-202.8 mg $KOH.g^{-1}$) that Hippola A *et al*. reported for *M. longifolia* **[22]**. The results of the experiment conducted by Chakraborty A and Ghosh DM found that *A. auriculiformis* seed oil (SV=193.8 mg $KOH.g^{-1}$) significantly higher than the SV of AFSO (145 mg $KOH.g^{-1}$) **[23]**. The degree of rancidity in oils is indicated by the AV and FFA values. Important metrics for characterizing and evaluating the quality of edible fats and oils are AV and FFA. Additionally, the FFA content is used to test for purity and, in some cases, to draw conclusions about the pretreatment or ongoing decomposition reactions. The seed oil's quality decreases with increasing AV and

FFA content. The MISO was found to have an AV of 48 mgKOH.g^{-1}, while the AFSO had an AV of 20 mgKOH.g^{-1}. MISO had a FFA value of 20.95% while AFSO had a FFA value of 0.6%, the oil is better suited for human consumption when the FFA level is lower. De-acidifying the MISO is necessary to reduce the FFA content; therefore the treated product would have better quality than the untreated MISO **[24]**.

Analysis of the antioxidant activity of MISO and AFSO

The antioxidant activity of MISO and AFSO were evaluated using the DPPH free radical scavenging method and IC_{50} values are shown in **Table 2**.

DPPH-free radical scavenging activity of MISO and AFSO

An efficient and quick way to assess antioxidant activity is with the DPPH radical scavenging assay. An electron or hydrogen radical can be taken up by the extremely stable organic free radical DPPH. As a result, absorbance decreases when antioxidants reduce DPPH. Accordingly, the degree of solution discoloration reveals the added substances' effectiveness at scavenging **[25]**. Table II displays the DPPH values of the studied seed oils and ascorbic acid (standard). Both MISO and AFSO interacted with and inhibited radical scavenging in terms of antioxidant activity. At all concentrations, MISO exhibited greater antioxidant activity than AFSO. Ascorbic acid concentrations ranged from 0.25 to 4µg/mL. Among the two studied seed oils, MISO exhibited the higher DPPH scavenging activity than AFSO. The IC_{50} value of the MISO was 4.54± 1.15 ug/mL, which is relatively close to the IC_{50} value of hemp seed oil (3.433 ± 0.017 ug/mL) as reported by Kalinowska *et al* **[26]**. Higher antioxidant potency is favored by a lower IC_{50} value **[27]**. Additionally, the AFSO has IC_{50} value (7.24± 1.19 mg/mL) is extremely closer to the values found for cherry seed oil (IC_{50}=7.68 ug/mL) and black cherry (IC_{50} = 7.28 ug/mL) as reported by Fratianni *et al* **[28]**. The DPPH scavenging assay of MISO and AFSO are shown in **Graphs 1 and 2**, respectively.

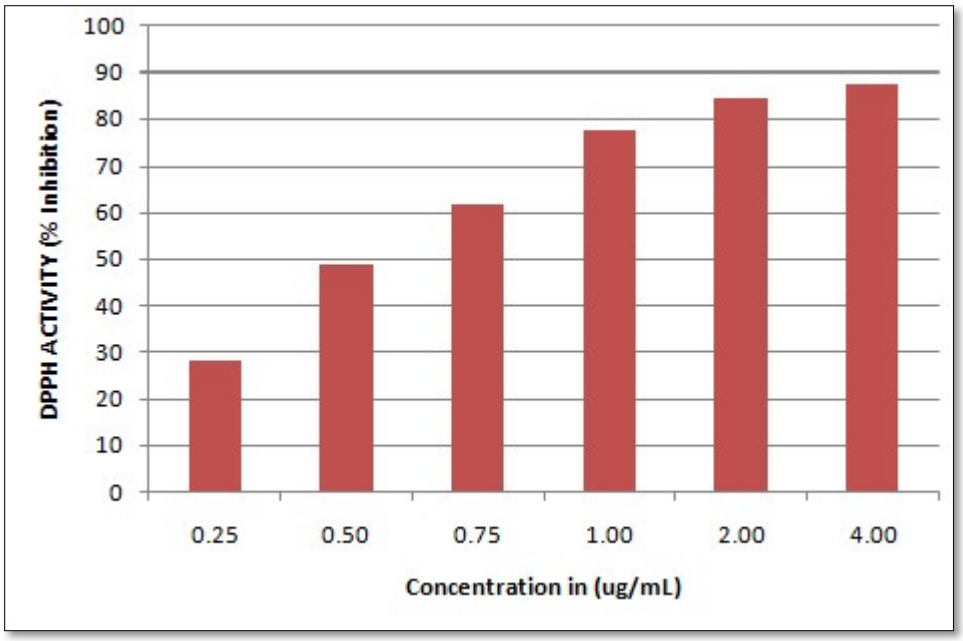
Table 1: Physicochemical Properties of MISO and AFSO

S. No.	Plant Species with family	Oil %	Density (Kg/m^3)	Protein %	Moisture %	SV (mgKOH. g^{-1})	IV (gI$_2$/100 g oil)	AV (mgKOH. g^{-1})	RI	FFA %
1.	*Madhuca indica* (Sapotaceae)	48.15	8.283	18.55	0.25	198.50	65	48	1.462	20.95
2.	*Acacia farnesiana* (Fabaceae)	25.77	8.456	20.75	0.6	145	110	20	1.467	0.6

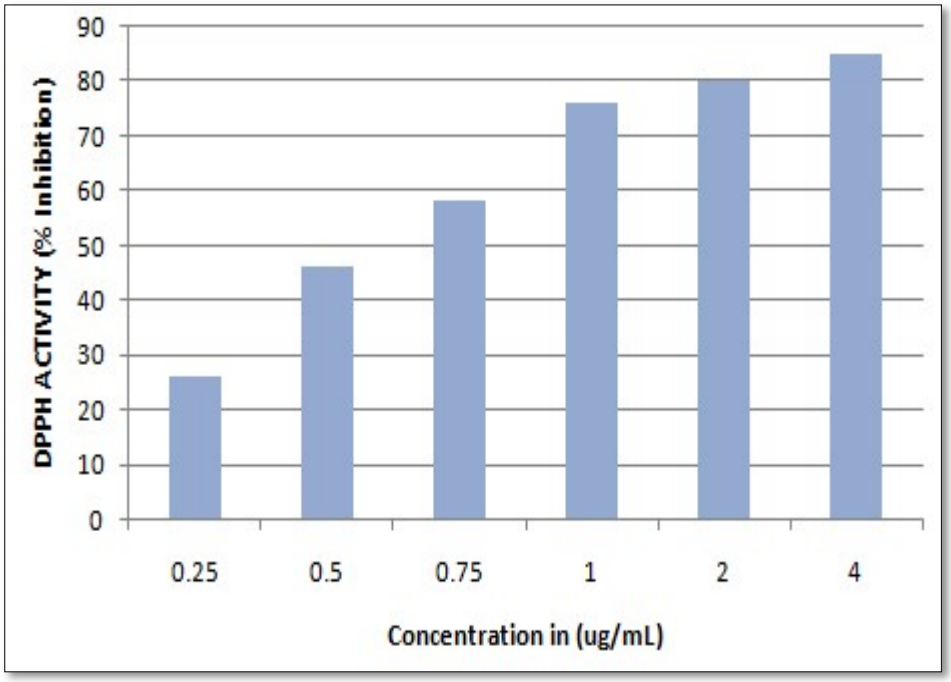
Table 2: DPPH Antioxidant at different concentrations in terms of IC_{50} of MISO, AFSO and ascorbic acid

Plant species	DPPH activity at different concentration ± SD (ug/mL)						
	0.25	0.5	.75	1.0	2.0	4.0	IC_{50} ± SD (ug/mL)
MISO	28±1.55	49±0.77	62±1.85	78±0.57	85±1.66	88±0.88	4.54± 1.15
AFSO	26±1.54	46±0.78	58±1.35	76±0.57	80±1.56	85±0.78	7.24± 1.19
Ascorbic acid	57.74±1.19	68±1.23	85±0,45	92±1.32	95±1.12	98±1.25	18.45±1.13

*** Values are given as Mean±SD (n=3)**



Graph 1: DPPH radical scavenging assay of MISO



Graph 2: DPPH radical scavenging assay of MISO

CONCLUSION:

The physicochemical analysis's findings demonstrated that the low IV of the AFSO and MISO both contribute to their oxidative stability. These seed oils under investigation have physicochemical characteristics that are in line with those of common edible seed oils. Furthermore, because of their lower IC_{50} values, the DPPH analysis of MISO and AFSO seed oils demonstrated that they are both good sources of naturally occurring antioxidants. However, toxicological testing must be done to ascertain the safety of both of these seed oils before they can be considered suitable for dietary use. To determine the bioactive component and assess the in vivo antioxidant potential of these seed oils, further investigation is required.

CONFLICT OF INTEREST: There is no conflict of interest.

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