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Effect of Solvent Polarity Index on Fatty acid, Phytochemical and Antioxidant Profiles of Oleoresin Extracts from *Monodora Myristica* Seed

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ABSTRACT: Oleoresin is a coloured viscous liquid composed of oils and resins extract from spice biomass, its proven suitability as industrial raw material with immense application, has stimulated increased search for this oleochemical with novel content. Hence, the objective of this paper was to assess the effect of solvent polarity index on fatty acid, phytochemical and antioxidant profiles of oleoresin extracts from *Monodora Myristica* seed using appropriate standard techniques. The Results showed TEC ranges from (4.54-10.20%) and present MMO_{ETH} oleoresin with the highest total extractable matter, TPC, TFC, DPPH activity, TAC, IRC and terpenes content characterized by monoterpenes and sesquiterpenes. Phlobatannins and Anthraquinones were present in MMO_{ETA} and MMO_{ETH} *M. Myristica* seed oleoresins *albeit* lower in the former. The study indicates preponderance levels of sterols and fatty acids contents in MMO_{PTE} relative to MMO_{CLF}, and also presents these oleoresins with absence of tannins, saponins, phlobatannins, anthraquinones and cardiac glycosides. 50% inhibitory concentration (IC_{50}) for TAC and DPPH radical scavenging activities presents MMO_{ETA} and MMO_{ETH} with greater antioxidant potential compared to ascorbic acid used as reference standard. The results showed that all the solvents were effective for extraction of oleoresin from *M. Myristica* seed with varied content suitable for recipes in food, non-food and pharmaceutical formulations.

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African nutmeg (*Monodora Myristica*) is a tropical tree of the family Annonaceae or custard apple family of flowering plants Okechukwu *et al.*, (2022). The tree is native to tropical Africa and grows naturally in evergreen forest in Southern-Nigeria and reaches a height of 35 m and 2 m in diameter Ajayi *et al.*, (2004). It has a clear trunk and branches horizontally, the leaves are alternately arranged and dropping with the leave blade being elliptical, oblong or broadest towards the apex and tapering to the stalk Onyenibe *et al.*, (2015), they are petiolate and can reach a size of 45 cm x 2 cm. fruits of *M. Myristica* are collected from wild trees, dried and sold whole or milled as ingredient for stews, soups, desserts and cakes Okechukwu *et al.*, (2022). In Nigeria *M. Myristica* seed enjoys the following native name; Gydan Miya (Hausa), Ariwo/Ariyo (Yoruba), Ehuru (Igbo), Ikposa (Benin) and Kposa (Ilaje) Onyenibe *et al.*, (2015). As medicine, the bark and fresh leaves of the tree find uses as insect repellants, stimulants and relief for stomach aches and headaches Adewole *et al.*, (2013). The milled seed powder as spice in soup to relieve

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constipation and control passive uterine hemorrhage in women immediately after child birth Ezenko *et al.*, (2017). The search for bioactive compounds from under-utilized native plants material to enhanced healthy living standards for mankind continues to receive desired attention from researchers in a steady manner. Non-nutritive phytochemicals compounds' possessing antioxidant properties which predominate leafy and non-leafy vegetables, legumes, seeds, fruits and in medicinal plants plays important role in reducing the risk of oxidative damage pose by free radicals released during metabolism Brewer, (2011); Nawaz *et al.,* (2020). Contributory factors namely; solvent polarity, solvent concentration, temperature and time has been established Nawaz *et al.,* (2020), to have a profound effects on the distinctive features of extract yield, phytochemical and antioxidants compounds derived from plant biomass. Solvent polarity can help tailor the chemical nature and constituents of extracted matter Ghasemzadeh *et al.*, (2015), as no single solvent can exhaustively remove all the phytochemicals and antioxidants contained in any plant biomass. Solvents with different polarity have been used sequentially in serial exhaustive extraction protocol, involving successive extraction with solvents of increasing polarity index Abdel-Aal *et al.* (2015).

Increase use of spice oleoresin extract from spice mass, instead of the native spice has gained more traction as innovation for food and non-food industrial applications. Uniform and concentrated flavor, economy of usage and absence of microbial contaminations are some of the merits of this oleochemical as industrial intermediates Samuel *et al.*, (2023). Oleoresins are not only indispensible food adjuncts, but find extensive application in cosmetics, nutriceutical, mobile phase for delivery of fat soluble vitamins, sources for dietary fatty acids and as drug depressants in therapeutics Ajayi *et al.,* (2004); Igwe *et al.,* (2005). Studies have reported the oil content of *M. Myristica* seed for domestic and industrial applications Ajayi *et al.,* (2004), Extraction, Physico-chemical, Phytochemical Analysis and Identification of some Important Compound in *M. Myristica* seed oil Ezenko *et al.,* (2017), Phytochemical Evaluation of Extracts and GC-MS analysis of *M. Myristica* seed oil Obonga *et al.,* (2019), Chemical Composition, Antibacterial Efficacy and Antioxidant Capacity of Essential Oil and Oleoresin from *M. Myristica* Seed Okechukwu *et al.,* (2022).

However, there is no documented report on the effect of solvent polarity index on the extraction yield, fatty acid, phytochemical and antioxidant contents of *M. Myristica* seed oleoresins. Furtherance, to the foregoing development, it's imperative to derive and evaluate the chemotypes of different oleoresin fractions from this underutilized plant biomass with a goal to harnessing its industrial potentials. Therefore, the objective of this paper was to assess the effect of solvent polarity index on fatty acid, phytochemical and antioxidant profiles of oleoresin extracts from *Monodora myristica* seed.

MATERIALS AND METHODS

Preparation of Monodora Myristica Seed: Monodora Myristica seeds were purchased from Zuru metropolis in Zuru Local Government Area of Kebbi State-Nigeria, the seed voucher was identity and authenticated by Dr. Hassan Ajayi Shindi of the Department of Crop Science, College of Agriculture, Federal University of Agriculture, Zuru. The seeds were decorticated to remove the reddish-brown kernels. The kernels were washed, sun dried for seven (7) days, grounded with a blender (Binatone, Japan, model BLG-400) and fractionated (<75 µm) to obtain fine powder, which was packed into a polyethylene bag and stored in a refrigerator.

Extraction of Monodora Myristica Seed Oleoresin: Oleoresin extracts were obtained by consecutive extraction method Nawaz *et al.,* (2020), with slight modification using a series of organic solvents with increasing polarity index (petroleum ether, chloroform, ethyl acetate and ethanol). 100 g of *M. Myristica* seed powder was weighed into a 500 ml volumetric flask, the flask was aggregated with 200 ml petroleum ether stopped and held on an orbital mechanical flask shaker (Innova 2000). The flask and its content were shaken for 24 h at ambient temperature, the flask was allowed to stand for 6 h, and the supernatant filtered to obtained (oleoresin + solvent). The oleoresin was recovered with Phoenix Instrument Rotary Evaporator (RE-100D) dried over silica gel for 48 h and weighed. The pomace obtained after extraction of oleoresin with petroleum ether, was weighed and further subjected to extraction consecutively in a serial order; chloroform, ethyl acetate and ethanol. In each extraction, the oleoresin was recovered from the solvent in a Rotary Evaporator, dried over silica gel for 48 h and weighed. The oleoresins were coded MMO_{PTE}, MMO_{CLF}, MMO_{ETA} and MMO_{ETH} for petroleum ether, chloroform, ethyl acetate and ethanol extracts respectively. Total Extractable Components was calculated, and the oleoresins were packed in amber bottles and stored in a refrigerator.

$$TEC\ (\%) = \frac{w}{W}\ X\ 100 \quad (1)$$

Where w is weight of oleoresin and W is the weight of *M. Myristica* seed mass before extraction

Fatty-acid Profile and Identification of Bioactive Compounds in M. Myristica Oleoresins: The GC-MS analyses of the oleoresin were conducted on a Hewlett- Packed HP 5973 mass spectrometer interfaced with an HP 6890 gas chromatograph. The following column and temperature conditions were used; initial temperature70 ^{o}C, equilibration time 3.00 min, ramp 4 ^{o}C min^{-1}, final temperature 240 ^{o}C; inlet: splitless, initial temperature 220 ^{o}C, pressure 8.27 psi, purge flow 30 ml min^{-1}, purge time 0.20 min, helium gas; column: capillary, 30 m x 0.25 mm i.d; 0.25 µm, film thickness 0.7 ml min^{-1}, average velocity 32 cm sec^{-1}; MS: EI method at 70 eV. The oleoresins compounds were identified by matching their mass spectra data with those of authentic standards held in the computer library (Wiley 275, New York) and by comparing the calculated retention indices with those in literature. The percentage composition was calculated from summation of the peak areas of the total oleoresins composition Asekun *et al.,* (2013); Samuel *et al.,* (2023).

Phytochemical Profile of M. Myristica Oleoresins: The methods Swapana *et al.,* (2012); Aziz *et al.,* (2021) was used to profile the oleoresin samples for terpenoids, tannins, saponins, steroids, flavonoids, cardiac glycosides, anthocyanins, phlobatannins, caroteniods, reducing compounds and anthraquinones.

Determination of Total Phenolic Content: The phenolic antioxidants present in the oleoresins were determined by Folin-Ciocalteu's method as previously described by Aziz *et al.,* (2021), Total Phenolic Content (TPC) were calculated as gallic acid equivalent g/100g dry weight from Gallic acid standard curve Nawaz *et al.,* (2020). The study was carried-out in triplicate; values reported are for the mean ± standard deviation.

$$TPC\ \left(\frac{g}{100g}dry\ wt.\right) = \frac{Absorbance\ at\ 720\ nm}{2.45} \quad (2)$$

Determination of Total Flavoniod Content: Total flavonoid content of *M. Myristica* seed oleoresin was determined by method as described by Okechukwu *et al.,* (2022), Total Flavonoid Content (TFC) were evaluated as quercetin equivalent g/100 g dry wt. using standard curve of quercetin in four different concentrations Nawaz *et al.,* (2020); Aiwonegbe and Ativie (2023). The study was carried-out in triplicate; values reported are for the mean ± standard deviation.

$$TFC\ \left(\frac{g}{100g}dry\ wt.\right) = \frac{Absorbance\ at\ 415\ nm}{4.64} \quad (3)$$

Determination of Free Radical Scavenging Activity: The free radical scavenging activity of oleoresins determined using 2, 2-diphenyl-2-picryhydrazyl (DPPH) as described by Aiwonegbe and Ativie (2023). A solution of 0.1 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 3.0 ml of oleoresin in methanol containing 0.001-0.05 mg/ml of oleoresin. The reaction was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance was determined at 517 nm with ascorbic acid as standard; the study was carried-out in triplicate. The radical scavenging activity (RSA) was calculated;

$$RSA\ (\%) = \frac{Abs\ (DPPH) - Abs\ (OLR)}{Abs\ (DPPH)} \quad (4)$$

Abs (DPPH) Absorbance of DPPH at 517 nm and Abs (DPPH) Absorbance of oleoresin at 517 nm

50% inhibitory concentration value (IC_{50}) was calculated as the effective concentration of the oleoresin that is required to scavenge 50% of the DPPH free radicals.

Determination of Reducing Power of Oleoresin: The ability of oleoresin to reduce Fe^{3+} to Fe^{2+} was evaluated as described by Aziz *et al.,* (2021), 2ml of oleoresin sample was aggregated with 2.5 ml of sodium phosphate buffer [2.5 ml 1% $K_3Fe\ (CN)_6$]. The mixture was incubated for 25 minutes at 50^{o} C, 2.5 ml of 10% trichloroacetic acid was added and centrifuged (2500 rpm for 20 minutes). 5ml of the supernatant was added to 0.5 ml 0.1% iron (III) chloride and 2.5 ml of distilled H_2O. The absorbance reading was taken with Gallic acid used as standard reference compound; determination was done in triplicate.

Determination of Total Antioxidant Capacity: Total antioxidant capacity of *M. Myristica* seed oleoresins was determined by phosphomolybdate assay as described by Igwe *et al.,* (2005); Aziz *et al.,* (2021), 1 ml of Molybdate reagent solution (4 Mm Ammonium Molybdate, 0.6 M H_2SO_4 and 28 mM Sodium Phosphate) was added to 0.1 mL of oleoresin sample in a test-tube. The tube was stopped and placed in a water bath at 85^{o} C for 90 minutes and cooled to ambient temperature, before measuring its absorbance at 765 nm with ascorbic acid used as standard reference compound the study was done in triplicate. Total Antioxidant Capacity was calculated;

$$TAC\ (\%) = \frac{Abs\ (CTRL) - Abs\ (OLR)}{Abs\ (CTRL)}\ X100 \quad (5)$$

Abs (CTRL) is the Absorbance of control at 765 nm and Abs (OLR) is the Absorbance of oleoresin at 765 nm

The concentration of oleoresin at which 50% (IC_{50}) inhibitory activity occurred was determined.

RESULTS AND DISCUSSION

The total extractable component, total phenolic content, total flavonoid content, of *M. Myristica* Seed Oleoresins is presented in table 1. The total extractable component shows MMO_{ETH} with the highest total extractable component, which may suggest that the highest solvent polarity index (5.1) for ethanol Nawaz *et al.,* (2020), enabled the extraction of more polar compounds, due to their solubility in this protic solvent. The low yield of 4.54% for MMO_{PTE} may be adduced to the lowest solvent polarity index (0.1) for petroleum ether, as low polarity functional groups easily dissolves in aprotic solvents Aziz *et al.,* (2021). Phenols and its derivatives functions as antioxidants, and offers protection against oxidative stress and inflammation caused by air-borne matter Aiwonegbe and Ativie (2023). Flavonoids do not only find applications in personal care products, but are essential dietary component to prevent metabolic disorders, cancer and as anti-proliferative agent Sinan *et al.,* (2021). The high phenolic and flavonoid contents of ethyl acetate and ethanol oleoresin extracts of *M. Myristica* seed presents these oleochemicals as rich sources of these bioactive compounds and attest to their applications as resins in food and pharmaceutical recipes. The TPC and TFC values for MMO_{ETH} and MMO_{ETA} are in concordance with those as reported for ethyl acetate and butanol extracts Obonga *et al.,* (2019) and the ethanol extract from this study is slightly lower than that for hydrodistillation Okechukwu *et al.,* (2022). The higher polarity and corresponding dipole moment of water compared to ethanol may be attributed to former extracting more of these polar compounds from *M. Myristica* seed. Tannins, saponins, cardiac glycosides, phlobatannins and anthraquinones, which were absent in MMO_{PTE} and MMO_{CLF} but, present with other phytochemicals in MMO_{ETA} and MMO_{ETH} *albeit* preponderant in the latter as shown in table 2, may be ascribed to higher polarity index for ethanol compared to ethyl acetate, as these bioactive compounds readily dissolves more in solvents with increased dipole moment Gorgani *et al.,* (2017).

Table 1: TEC, TPC, TFC of *M. Myristica* Seed Oleoresins

Oleoresin	TEC (%)	TPC (g/100 g dry wt.)	TFC (g /100 g dry wt.)
MMO_{PTE}	4.54	18.51±2.31	10.22±1.44
MMO_{CLF}	4.91	34.12±3.25	14.11±2.28
MMO_{ETA}	7.68	48.77±4.45	67.15±3.24
MMO_{ETH}	10.20	53.13±2.18	84.29±5.12

Phlobatannins and Anthraquinones have been identified with profound and remarkable antioxidant activities Nawaz *et al.,* (2020). Phlobotannins are C-ring isomerized oligomeric flavonoids compounds with elevated antioxidant capacity compared to polyphenol, and have shown antioxidant activities notably; reduction of oxidative stress, anti-inflammation and anti-tumor Swapana *et al.,* (2012). Anthroquinones constitute a large structural variety of compounds composed of two aromatic rings joined by two carbonyl groups to form a planar aromatic structure among the polyketide group, that finds application as dye precursor for water-soluble dye that readily impregnates fibre and textiles, anthracycline family of chemotherapy drugs, industrial production of hydrogen peroxide and its sodium-2-anthraquinonesulfonate derivative as an effective catalyst in alkaline pulping processes Stalman *et al.,* (2003). As medicine exhibit laxative, diuretic, estrogenic and immunomodulatory effects and excellent ability to combat free radicals, effectively coordinate metals both *in vitro* and *in vivo* and maintaining cognitive function Aiwonegbe and Ativie (2023).

Table 2: Phytochemical Profile of *M. Myristica* Seed Oleoresins

Phytochemical	Oleoresin			
	MMO_{PTE}	MMO_{CLF}	MMO_{ETA}	MMO_{ETH}
Terpenoids	++	++	++	+++
Tannins	-	-	+	++
Saponins	-	-	++	++
Steroids	+	++	-	-
Flavonoids	+	+	+	++
Cardiac glycosides	-	-	+	+++
Anthocyanins	+	+	+	++
Phlobatannins	-	-	+	++
Caroteniods	+	+	+	++
Reducing compounds	+	+	+	++
Anthraquinones	-	-	+	+

(-)=absent, (+) = available in small amount, (++) =available in moderate amount, (+++) =available in large amount

Table 3: Fatty acid and Bioactive components of *M. Myristica* seed oleoresins

Compound	Composition (%)			
	MMO_{PTE}	MMO_{CLF}	MMO_{ETA}	MMO_{ETH}
α-lemonene	-	-	1.22	2.71
Germacrenes	-	-	0.92	1.44
Eicosadienoic acid	4.85	2.07	-	-
Eicosatrienoic acid	3.42	1.00	-	-
Linoceric acid	1.13	-	-	-
Arachidic acid	6.27	1.65	-	-
Gadoleic acid	2.53	-	-	-
Stearic acid	4.10	-	-	-
Palmitic acid	5.54	-	-	-
Oleic acid	30.54	2.40	-	-
Linoleic acid	31.27	3.23	-	-
1,2-(Methylenecyclopropyl) Cyclopentene	-	-	-	3.22
2,4-Dimethyl-1,3-Cyclopentanedione	-	-	1.35	3.34
2-Acetylcyclopentanone	-	5.81	10.44	64.60
α-phellendrene	-	-	0.71	5.83
δ-Cadinene	-	-	0.58	0.94
2-Carene	-	-	0.55	1.68
α-pinene	-	-	0.52	2.00

The presence of these special classes of antioxidants in these oleoresins corroborates their high total phenolic content and total flavonoids content, relative to MMO_{PTE} and MMO_{CLF}. Results from table 2 indicate MMO_{ETH} oleoresin extract contained more polar antioxidants and reducing compounds, which substantiate its higher total phenol and total antioxidant contents as evidenced in table 1. Although moderately rich in terpenoids and Steroids MMO_{PTE} and MMO_{CLF} oleoresins shows low total phenol and flavonoids contents. The high content of phenols and flavonoids in oleoresin extracted with ethanol is in close range with those reported for methanol and aqueous extracts Obonga *et al.,* (2019).

The fatty acid profile and other bioactive components of the brownish slightly viscous liquid *M. Myristica* seed oleoresins at room temperature are presented in table 3. Linoleic (31.54%) in MMO_{PTE}, and (3.23%) in MMO_{CLF} was the predominant polyunsaturated fatty acid; oleic (30.54%) in MMO_{PTE}, and (2.40%) in MMO_{CLF} was the monounsaturated fatty acid. The saturated fatty acid presents were arachidic (6.27%) in MMO_{PTE}, (1.65%) in MMO_{CLF}, palmitic (5.54%) in MMO_{PTE}, and stearic (4.10%) in MMO_{PTE}. Clearly, the high percentage of fatty acids in MMO_{PTE} compared to MMO_{CLF} is not unconnected with the lower polarity index of (0.1) for petroleum ether relative to (4.1) for chloroform Ghasemzadeh *et al.,* (2015), as solubility of triglycerides esters and solvent polarity index shows an inverse relation Eromosele and Eromosele, (2002). Thus, corroborate MMO_{PTE} oleoresin with high valued dietary fatty acid as represented by linoleic (polyunsaturated fatty acid), oleic (monounsaturated fatty acid), Eicosadienoic acid (omega-6-fatty acid) and Eicosatrinoic acid (omega-3-fatty acid), and stand this oleoresin in good stead as ingredient in nutriceutical and functional foods, owing to the effects of these lipids molecules to lower serum cholesterol and mitigate coronary disease Ajayi *et al.,* (2004); Adewole *et al.,* (2013). Saturated fatty acids notably; palmitic and myristic have been reported in the narrowing of coronary arteries by deposition of triglyceride matter on the arterial wall, which may result in thrombosis Oshodi *et al.,* (1995). The low profile of palmitic acid (5.54%) and absence of myristic acid present these *M. Myristica* seed oleoresin, without dietary risk factor of reduced blood flow to the heart muscle and deprives it of oxygen, which may result to myocardial infarction Bender, (1992); Oshodi *et al.,* (1995). Besides, the low content of saturated fatty acid in MMO_{PTE} oleoresin, suggests its stability to oxidative rancidity, suitability for stir-frying and high smoke point Samuel *et al.,* (2013). The high component of fatty acids and steroids in MMO_{PTE} and MMO_{CLF} suggests the presence of α-tocopherols a fat-soluble antioxidants and its isomers in these oleoresins.

The ethyl acetate and ethanol *M. Myristica* seed oleoresins extract shows preponderance of monoterpenes and sesquiterpenes. The presence of these bioactive compounds comes with associative health and allied benefits; as they promotes weight loss, treat bronchitis, possesses antimicrobial and insecticidal properties Obonga *et al.,* (2019). Chemo-types namely; α-phellendrene α-pinene, α-lemonene, δ-Cadinene, Germacrenes, 2, 4-Dimethyl-1, 3-Cyclopentanedione, 1, 2-(Methylenecyclopropyl) Cyclopentene and 2-Acetylcyclopentanone present in MMO_{ETA} and MMO_{ETH} *albeit* higher in the latter may be ascribed to the amphiprotic character of ethanol. Reports have shown the preponderance of these chemo-types in oleoresin extracted with ethanol Brewer, (2011); Ghasemzadeh *et al.,* (2011); Ezenko *et al.,* (2017).

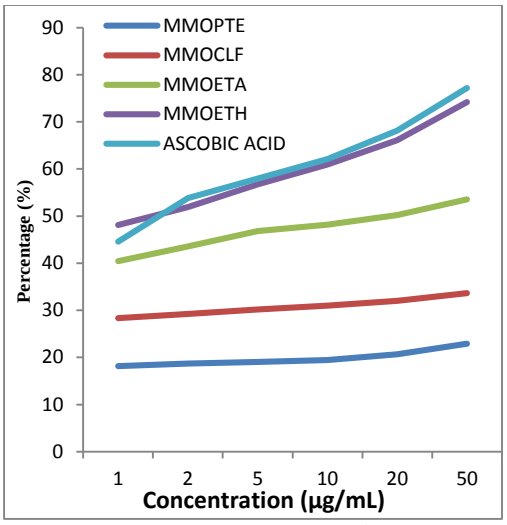

Fig 1: Radical Scavenging Activity (RSA) of *M. Myristica* seed oleoresins

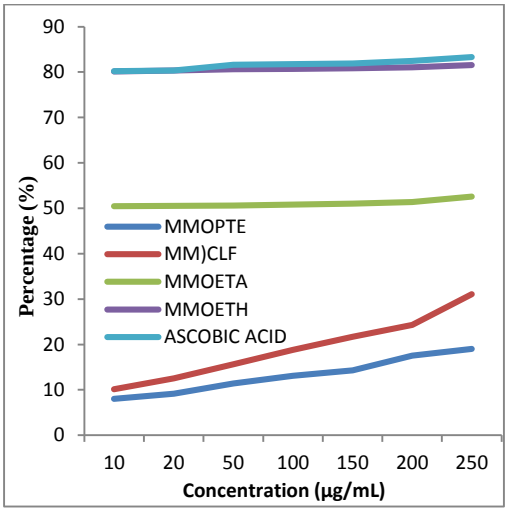

Fig 2: Total Antioxidant Capacity (TAC) of *M. Myristica* seed oleoresins

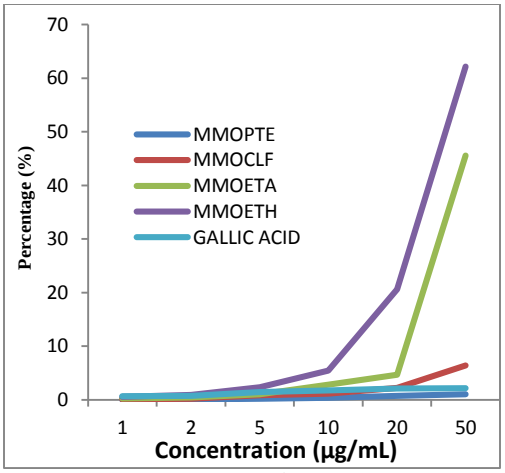

Fig 3: Iron Reducing Power (IRC) of *M. Myristica* seed oleoresins

The free radical scavenging potential of *M. Myristica* seed oleoresins at concentrations 1-50 µg/ml are shown in figure 1. Antioxidants are molecules that scavenge free radicals produced from perturbed mitochondrial respiratory processes that are harmful to the body Liu *et al.*, (2023). The result demonstrates a concentration dependent radical scavenging effects for the oleoresins. MMO_{ETH} did not only presents with the highest DPPH assay, but have values comparable to that of ascorbic acid as shown by the convoluted character of their curves. This *in vitro* antioxidant assay shown by MMO_{ETH} and MMO_{ETA} oleoresins represents the distinctive character of ethyl acetate and ethanol as preferred solvents for extraction of polar compounds from *M. Myristica* seed biomass. This finding is consistent with previous studies Igwe *et al.*, (2005); Okechukwu *et al.*, (2022). The highest radical scavenging activity demonstrated by MMO_{ETH} compared to other oleoresins confirmed ethanol extract with more potent antioxidants and affirms previous studies Gorgani *et al.*, (2017); Aziz *et al.*, (2019). The significant influence of Cardiac glycosides and phenolic amides as antioxidants Singh *et al.*, (2013), suggest the highest concentration of these typical antioxidants in MMO_{ETH}. The antioxidant scavenging activities as shown by petroleum ether and chloroform *M. Myristica* seed oleoresin extracts could be attributed to the presence of α- tocopherols and its isomers in these oleochemicals, owing to the solubility of these 16-carbon phytyl chromanol ring antioxidants in lipids Liu *et al.*, (2023). Clearly, this concentration-dependent DPPH antioxidant assay *M. Myristica* seed oleoresins, is indicative of a strong and effective antioxidant properties of these oleochemicals at higher concentrations.

Figure 2 presents the total antioxidant capacity of *M. Myristica* seed oleoresins as determined by molybdate assay. This assay is based on the reduction of molybedeum Mo^{6+} to Mo^{5+} by the oleoresins, followed by formation of green phosphate-molybdate (Mo^{5+}) complex at an acidic p^H. Velioglu *et al.*, (1998). The assay shows highest values for MMO_{ETH} oleoresin which is in concordance with values for ascorbic acid, as evidenced by the contiguous nature of their curves, due to marginal difference in their total antioxidant values within the concentration range (10-250 µg/ml). This suggests that the least concentration (10µg/ml) of MMO_{ETH} oleoresin has similar antioxidant potential with ascorbic acid.

The reducing powers of the oleoresins are presented in figure 3. The reducing power increases with increase in concentration within the range (1-50 µg/ml). The reducing power of oleoresins, take place largely because the reductones can transfer electrons or

hydrogen atoms that break the free radical chain Aziz *et al.,* (2021); Okechukwu *et al.,* (2022). The highest reducing power above 20 µg/ml concentration as shown by MMO_{ETH} and MMO_{ETA} *albeit* greater for the former which out-class that for Gallic acid, implies that beyond this concentration threshold, the antioxidants in these *M. Myristica* seed oleoresins, acting as a defense mechanism against lipid peroxidation would bind and coordinate to metal ions by reducing them to other variable oxidative species more readily than Gallic acid. This results as evidenced in figure 3, which is consistent with IC_{50} for these oleoresins as shown in table 4 , indicates the anti-oxidative potency of these oleoresins are manifold greater than that for Gallic acid.

Table 4: Inhibitory concentration (IC_{50}) of *M. Myristica* Seed Oleoresins

Oleoresin	IC_{50} (µg/ml)	
	TAC	DPPH
MMO_{PTE}	148.11	4.78
MMO_{CLF}	137.24	4.23
MMO_{ETA}	83.14	2.64
MMO_{ETH}	38.35	1.43
Ascorbic acid	142.02	4.46

The DPPH and total antioxidant capacity at 50% inhibitory concentration are shown in table 4. The values 38.35-148.11 µg/ml and 1.43-4.78 µg/ml, for TAC and DPPH respectively, present TAC values for MMO_{PTE} and MMO_{CLF} in close range with that for ascorbic acid. Suggest that the anti-oxidative properties of these oleoresins, presumably by α-tocopherols and its isomers are similar to that for ascorbic acid. The DPPH radical scavenging for MMO_{ETA} and MMO_{ETH} with values 2.64 and 1.43 suggest these oleoresins are two and three-folds respectively, more potent than ascorbic acid and could be attributed to the presence of phlobatannins (C-ring isomerized oligomeric flavonoids) and anthraquinones with elevated anti-oxidative merits in these oleoresins. In inflammatory process, the production of excess reactive oxygen species (ROS) as a result of imbalance of biological antioxidants could result to oxidative stress Hakim, (1993).

Moreover, reactive oxygen metabolites from phagocytic leucocytes may invade body tissues, causing injury to cells Silva *et al.,* (2005). Therefore, these *M. Myristica* seed oleoresins, judging by their IC_{50} values for DPPH radical scavenging activity (RSA) and total antioxidants capacity (TAC), stand them in good stead to scavenge free radicals, more than the reference compound as they could play significant role as sources for more potent antioxidants in the treatment and management of inflammatory disorders.

Conclusion: Oleoresin with varied contents have been extracted from *M. Myristica* seed using solvents with different polarity index by consecutive extraction in a series of four solvents with increasing polarity index. The results showed that *M. Myristica* seed oleoresins are endowed with dietary bioactive compounds and content of extracts were influenced by solvent polarity index. It can be concluded that *M. Myristica* seed is an esoteric pharmaceutical and food adjuncts with auspicious potential for industrial oleochemicals. It worthy to state that the research team has initiated application of spectroscopic tools notably; ^{1}H and ^{13}C Nuclear Magnetic Resonance NMR and Fourier-Transform Infrared to elucidate the structural make-up of the bioactive components of each extract and will be reported in due course.

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Data Availability Statement: Data are available upon request from the first author or corresponding author.

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