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Phytochemical Analysis and Free Radical Scavenging Efficacy of Novel Flavonoid Derivatives from *Euphorbia neriifolia and Mentha spicata leaves*

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Abstract: ***Background:*** Recent interest has focused on free radical chemistry. Due to endogenous activities, physiochemical circumstances, and pathologies, our bodies create reactive oxygen and nitrogen species and free radicals. When the body's free radical defenses fail, oxidative stress occurs. Free radicals destroy lipids, proteins, and DNA, causing many diseases. Thus, exogenous antioxidants can reduce oxidative damage. E. neriifolia is used as a laxative, purgative, rubefacient, carminative, expectorant, and to treat whooping cough, gonorrhoea, leprosy, asthma, dyspepsia, and jaundice. Mentha spicata, commonly known as Mentha viridis, is a Lamiaceae medicinal plant with antioxidant, anticancer, antiparasitic, antibacterial, and antidiabetic activities. ***Methodology:*** Extracts indicating the presence of flavonoids will undergo column chromatography for the further isolation of specific chemicals. The antioxidant capacity was further assessed using the DPPH test. ***Result:*** Findings indicated that the hydroalcoholic extracts from both plants contained the most distinct phytochemical substances. Among the evaluated substances, quercetin exhibited the greatest percentage of inhibition in both plant extracts relative to the standard. ***Conclusion:*** The findings demonstrate that both plants are excellent sources of natural antioxidants and underscore the therapeutic significance of flavonoid molecules.

Keywords: Euphorbia neriifolia, *Mentha spicata*, Phytochemical screening& antioxidant activity.

Research Paper

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INTRODUCTION

Antioxidant is an abbreviation for "against oxidation. An antioxidant is a substance that, in minimal quantities relative to an oxidizable substrate, significantly inhibits or halts the oxidation of that substrate. Antioxidants are crucial for maintaining food quality and promoting human health [1]. The location of the oxidation process determines the resultant consequences. Food deteriorates if the food system is the locus of the occurrence. In biological cellular systems, oxidation leads to cellular damage or mortality. The presence of fats and oils as food ingredients leads to oxidative deterioration, resulting in the loss of flavor and fragrance, hence diminishing nutritional value, sensory appeal, and safety [2]. This arises from the auto-oxidation of unsaturated fatty acids via a free radical chain mechanism, yielding initial hydroperoxides and subsequent potentially harmful compounds. The direct oxidation of unsaturated lipids, characterized by a double bond in a singlet state, by oxygen in its ground triplet state is spin forbidden, as the former possesses paired electrons in the same orbital with opposite spins, while the latter has two free electrons in separate orbitals with identical spin direction [3]. Cancer and cardiovascular disease are often induced by oxidative stress and an imbalance in antioxidant properties. Natural antioxidants such as ascorbic acid and tocopherols have demonstrated protective effects against cancer and cardiovascular disease. Cereals, vegetables, fruits, oilseeds, legumes, cocoa products, drinks (such as tea, coffee, red wine, beer, and fruit juices), herbs, and spices are the primary sources of natural antioxidants [4]. The utilization of antioxidants as functional dietary components and nutritional supplements, whether natural or synthetic, is progressively becoming more popular. Synthetic antioxidants are utilized in the food industry to stabilize fats, oils, and lipids, as well as in the pharmaceutical

sector and as preservatives in cosmetics, owing to their accessibility and heightened activity [5-8]. *Euphorbia neriifolia*, also known as the Indian Spurge tree or Hedge Euphorbia, is a species within the Euphorbia genus, recognized for its extensive local therapeutic use in the regions where it is cultivated. All possess latex and a distinctive floral architecture. A considerable proportion is succulent, mostly sourced from Africa and Madagascar [9]. Plants serve as bitter laxatives, carminatives, enhance appetite, and are beneficial for gastrointestinal disorders, bronchitis, tumors, leucoderma, hemorrhoids, inflammation, splenomegaly, anemia, ulcers, fever, and chronic respiratory conditions. A study revealed that E. neriifolia leaf extract had significant analgesic, anti-inflammatory, moderate central nervous system depressant, wound healing, and both humoral and cell-mediated immunostimulating properties. E. neriifolia decreased blood lipid levels and glucose, indicating its catabolic properties [10]. Mentha spicata, commonly known as Mentha viridis, is a medicinal plant belonging to the Lamiaceae family, distinguished by its ability to manufacture and secrete secondary metabolites, mostly essential oils. Various populations utilize the aerial components of this plant for tea preparation, and this tisane has demonstrated multiple effects, as indicated by ethnopharmacological studies conducted in diverse global regions. The effects are ascribed to several chemicals of M. spicata, whose biological effects were recently demonstrated experimentally. The pharmacological activities of M. spicata extracts and essential oils were examined for several health advantages, including antioxidant, anticancer, antiparasitic, antibacterial, and antidiabetic actions [11].

Euphorbia neriifolia

M. spicata

The current study was conducted to screen the phytoconstituents of different extracts of *Euphorbia neriifolia and M. spicata.* Subsequent isolation of new flavonoid derivatives was performed using column chromatography, followed by assessment of their free radical scavenging activity.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The procurement and validation of plant material are crucial preliminary steps in ensuring the quality and consistency of pharmacognostic and phytochemical studies. This study utilized fresh leaves of Euphorbia neriifolia sourced from local medicinal plant nurseries and verified growing locations. Dr. Smruti Sohani, a licensed botanist and Professor at SAGE University in Indore, Madhya Pradesh, meticulously examined the collected specimens for their morphological characteristics and verified their genuineness. The voucher specimen [PA-ENL-012] was subsequently preserved for future reference.

Extraction Procedures

The powdered plant material will undergo extraction with solvents of ascending polarity, including petroleum ether, chloroform, ethyl acetate, and a hydroalcoholic solvent. Extraction may be conducted via either Soxhlet equipment or the maceration process, contingent upon appropriateness. The acquired extracts will be concentrated utilizing a rotary evaporator to eliminate the solvent. The % yield of each extract will be computed and documented.

Phytochemical Assessment

The produced extracts will be evaluated for the presence of significant secondary metabolites, including flavonoids, alkaloids, tannins, glycosides, saponins, and phenolic compounds, utilizing established qualitative assays. Specific assays, including the Shinoda test and alkaline reagent test, will be conducted to verify the presence of flavonoids in plant extracts [12-13].

Column Chromatography Separation

Extracts demonstrating the presence of flavonoids will undergo column chromatography for the further separation of specific components. Various solvent systems will be employed to elute chemicals from the column. Fractions obtained from the column will be analyzed by TLC to identify those rich in flavonoids.

Isolation and Purification of Flavonoids

Fractions exhibiting analogous TLC patterns will be amalgamated and subsequently purified to separate flavonoid chemicals. The purity of isolated chemicals will be verified using chromatographic methods [14]. Flavonoid derivatives quercetin,

kaempferol and luteolin were isolated from both plant leaf hydroalcoholic extract.

Antioxidant Activity

The antioxidant activity of isolated flavonoid compounds derived from Euphorbia neriifolia and Mentha spicata leaves was assessed to ascertain their capacity to neutralize free radicals. Flavonoids are recognized natural antioxidants owing to the presence of phenolic hydroxyl groups and a conjugated aromatic ring structure. These structural characteristics allow flavonoids to transfer hydrogen atoms or electrons to free radicals, thus stabilizing them and averting cellular harm. The antioxidant activity of isolated compounds was assessed using the DPPH free radical scavenging test. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a persistent free radical that exhibits a rich violet hue in solution. Upon the addition of an antioxidant chemical, the DPPH radical takes a hydrogen atom from the antioxidant and is reduced to a yellow-colored product. The reduction in absorbance signifies the scavenging action of the test chemical. The absorbance of the reaction mixture was measured at 517 nm utilizing a UV-Visible spectrophotometer. The percentage suppression of free radical activity was determined using a conventional formula. A greater percentage of inhibition signifies a more robust antioxidant capacity of the chemical [15].

Formula for% inhibition

$$\text{Percent Inhibition} = \left(\frac{C - S}{C}\right) \times 100$$

Where: C is the control activity (the activity of the control sample) S is the sample activity (the activity of the sample sample).

RESULT AND DISCUSSION

Oxidative stress (OS) refers to an imbalance between the production of reactive oxygen species (ROS) and the efficacy of antioxidant defense systems. At elevated quantities, reactive oxygen species (ROS) can interact with many macromolecules, hence contributing to several disease processes. The cellular antioxidant defense mechanisms in our body, including glutathione (GSH) and reactive oxygen species (ROS) scavenging enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX), control ROS levels. Plants have always served as a source of optimism for story therapeutic molecules, since herbal mixes derived from them have significantly contributed to human health and well-being. This work was undertaken to examine the preliminary phytochemical analysis, isolate new flavonoid derivatives, and elucidate the antioxidant activity, taking into account the ethnopharmacological usage of both plants. The phytochemical examination of leaf extracts from Euphorbia neriifolia and Mentha spicata, utilizing petroleum ether, chloroform, ethyl acetate, and hydroalcoholic solvents, revealed an uneven distribution of bioactive chemicals. The petroleum ether extract of Euphorbia neriifolia mostly exhibited terpenoids, steroids, and fixed oils. The chloroform extract demonstrated positive findings for alkaloids, terpenoids, and glycosides. The ethyl acetate extract included flavonoids, phenolic chemicals, and tannins. The hydroalcoholic extract included several phytochemicals, including alkaloids, flavonoids, tannins, glycosides, saponins, and carbohydrates. The petroleum ether extract of Mentha spicata mostly included terpenoids, essential oils, and fixed oils. The chloroform extract included alkaloids, terpenoids, and flavonoids. The ethyl acetate extract included flavonoids, phenolic chemicals, and tannins. The hydroalcoholic extract exhibited a distinctive phytochemical profile, characterized by a high concentration of alkaloids, flavonoids, phenolics, and carbohydrates, with a little presence of saponins. The hydroalcoholic extract of Euphorbia neriifolia only included proteins and amino acids, which were absent in Mentha spicata. The hydroalcoholic extracts from both plants exhibited the most distinct phytochemical profiles. This indicates that this solvent solution is more effective at extracting many polar or semi-polar bioactive molecules. These findings provide a phytochemical foundation for next pharmacological and therapeutic investigations. The result was tabulated in table 1& fig 1. We employed solvent fractionation, column chromatography, and thin layer chromatography (TLC) to get the flavonoid compounds out of the leaves of Euphorbia neriifolia and Mentha spicata. The goal of separation was to get rid of the parts of crude extracts that had a lot of flavonoids in them. This would make it easy for them to apply spectral approaches in their research. Flavonoid derivatives quercetin, kaempferol and luteolin were isolated from both plant leaf hydroalcoholic extract. The antioxidant efficacy of isolated flavonoid compounds derived from E. neriifolia and M. spicata was assessed using the DPPH radical scavenging test. The results indicated that all isolated compounds demonstrated meaningful consequences. Quercetin exhibited the greatest percentage inhibition (91.24 ±1.08) among the substances, with an IC50 value of 24.35 ±0.64 µg/ml, in comparison to the standard ascorbic acid. Kaempferol and luteolin demonstrated significant antioxidant activity with modest IC50 values (table & fig. 2-3). The presence of many hydroxyl groups in the flavonoid structure accounts for its high antioxidant action. The radical scavenging capacity is contingent upon the quantity and placement of hydroxyl substituents in the aromatic ring. The results indicated that flavonoid derivatives extracted from both plants may serve as effective natural antioxidants.

Table 1: Preliminary phytochemical screening of successive solvent extracts of Euphorbia neriifolia and Mentha spicata leaves

Phytochemical	PEEE NL	CEENL	EAEE NL	HAEE NL	PEEM SL	CEMS L	EAEM SL	HAEM SL
Carbohydrates				+	-I			+
Alkaloids		+		+		+		+
Flavonoids	+	+	+	+		+	+	+
Phenolic compounds & Tannins	-	-	+	+	-	-	+	+
Saponins	-	-	-	+	-	-	-	+
Glycosides	-	+	-	+	-	-	-	-
Terpenoids	+	+	-	-	+	+	-	-
Steroids	+	-	-	-	-	-	-	-
Proteins & Amino acids		-	-	-	-	-	-	-

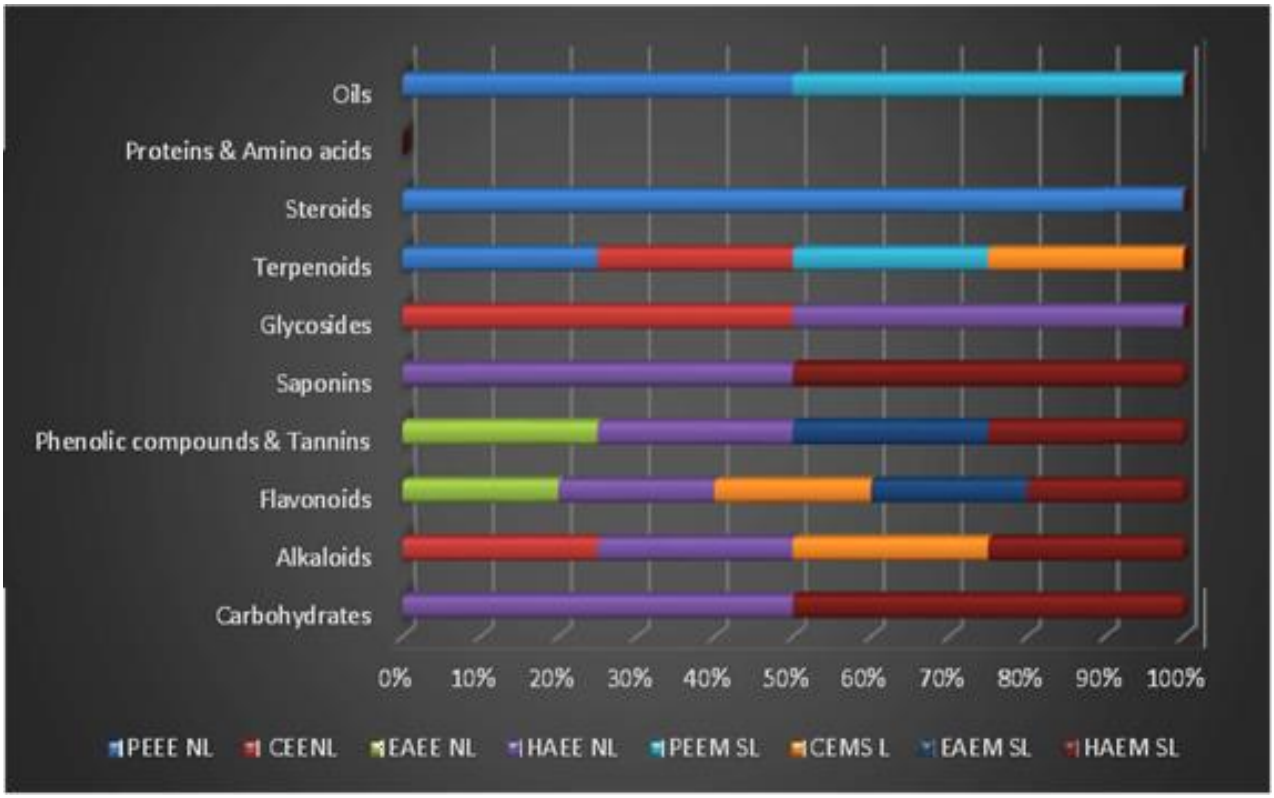

Fig. 1: Phytochemical profile of various extracts of Euphorbia neriifolia and Mentha spicata leaves

Table 2: DPPH Scavenging Activity of isolated Compound

Plant Source	Flavonoid Compound	% Inhibition at 100 µg/mL	ICso (µg/mL)
Euphorbia neriifolia leaves	Quercetin	91.24 ± 1.08	24.35 ± 0.64
	Kaempferol	84.17 ± 1.32	31.78 ± 0.81
	Luteolin	78.49 ± 1.45	39.26± 1.04
Mentha spicata leaves	Quercetin	94.85 ± 1.12	18.67 ± 0.55
	Kaempferol	89.73 ± 1.25	25.61 ± 0.72
	Luteolin	82.36± 1.41	33.49 ± 0.96
Standard (Ascorbic acid)	__	96.42 ± 1.10	15.45 ± 0.48

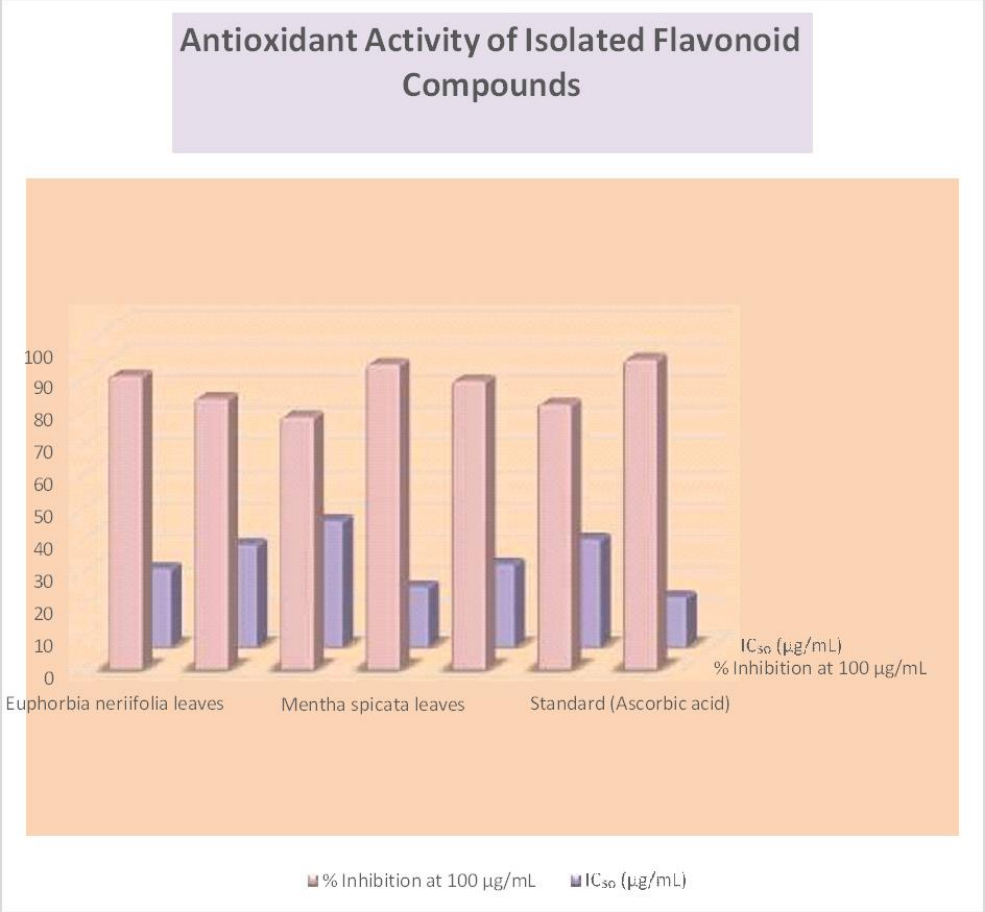

Fig. 2: Comparative antioxidant activity of isolated flavonoid derivatives by DPPH assay

Table 3: % inhibition of isolated flavonoid compound

Flavonoid Compound	% inhibition		
	E.nerifolia	M.spicata	Ascorbic acid
Quercetin	91.24 ± 1.08	94.85 ±1.12	96.42± 1.10
Kaempferol	84.17 ±1.32	89.73 ±1.25	96.42± 1.10
Luteolin	78.49 ±1.45	82.36 ±1.41	96.42± 1.10

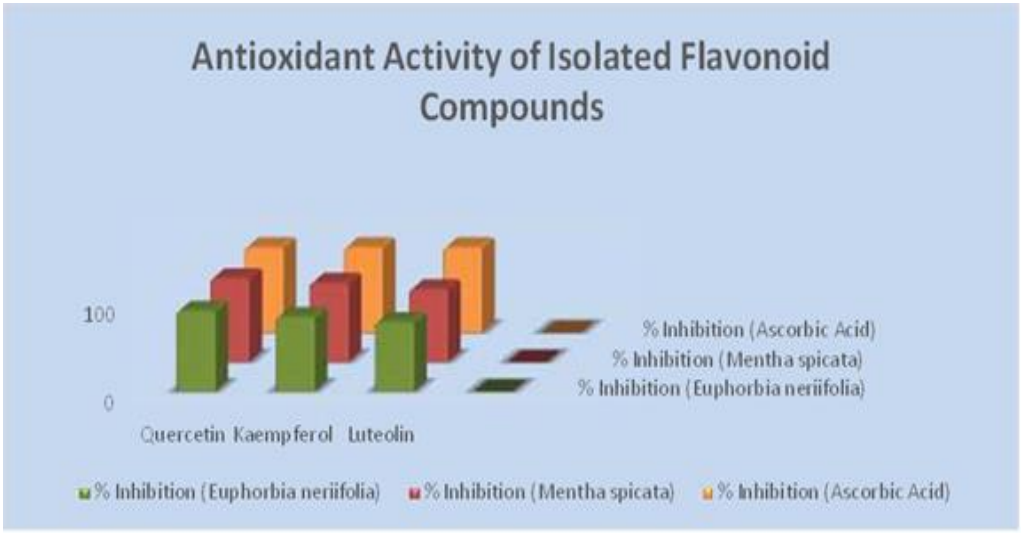

Fig 3: % inhibition of isolated flavonoid compound

CONCLUSION

The findings of the antioxidant activity demonstrated that all extracted flavonoid compounds displayed considerable DPPH radical scavenging activity. Of the substances evaluated, quercetin exhibited the greatest percentage of inhibition in both plant extracts. Mentha spicata had much more antioxidant activity than Euphorbia neriifolia. The standard medication ascorbic acid demonstrated the highest level of inhibition and served as the reference. Kaempferol and luteolin exhibited notable antioxidant capability, but with somewhat reduced efficacy relative to quercetin. The elevated antioxidant activity of quercetin may result from the presence of numerous hydroxyl groups in its chemical structure, which augment its capacity to donate hydrogen atoms and destroy free radicals. The findings demonstrate that both plants are excellent sources of natural antioxidants and underscore the therapeutic significance of flavonoid molecules.

Divulgence of Investigation

The presence of several hydroxyl groups imparts significant antioxidant, chelating, and prooxidant properties to the molecule. Methoxy groups impart adverse steric effects and enhance lipophilicity and membrane partitioning. The presence of a double bond and carbonyl group in the heterocycle or polymerization of the nuclear structure enhances activity by providing a more stable flavonoid radical via conjugation and electron delocalization.

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