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Phytochemical Studies of Curry (*Murraya koenigii*) and Eucalyptus (*Eucalyptus globulus*) Leaves Extracts

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ABSTRACT: In the traditional system of medicine *Murraya koenigii* and *Eucalyptus globulus* is important medicinal plant cultivated throughout India. The plants have a peculiar characteristic aroma and are used against the wide variety of ailments caused by several pathogenic entities. Every part of each plant has been medicinal values and is of economic important. The highest yield of aqueous and methanolic eucalyptus leaves extracts were 13.07% and 17.17%, respectively whereas for curry leaves extracts were 09.02% and 16.71%, respectively. The blackish and greenish brown colored extract was observed in aqueous and methanolic eucalyptus leaves, respectively whereas the brownish black and brown color extracts were seen in aqueous and methanolic curry leaves, respectively. The solid or powdery consistency was revealed in aqueous eucalyptus leaves and methanolic curry leaves extracts whereas the solid consistency was noted in methanolic eucalyptus leaves extract while semi-solid in aqueous curry leaves extract. The leaves extract of both plants constitutes various active ingredients like alkaloids, tannins, saponins, coumarin glycoside, flavonoids, triterpenes, steroids, essential oils etc. which facilitates further pharmacological studies. However, standardization, quality control, toxicity and drug interaction are the main challenges behind using herbal drugs. In this regard, the present work carried out study the preliminary phyto-chemical of the aqueous and methanolic leaves extracts of *Murraya koenigii* and *Eucalyptus globulus*. Thorough study need to be carried out for the identification and quantification of active chemicals and their judicious use in the treatment of various animal and human infections.

Keywords: *Eucalyptus globulus, Murraya koenigii*, Phyto-constituents, Traditional medicines.

INTRODUCTION

Since ancient time conventional medicines provide a source of essential therapy for a wide range of ailments especially in the developing countries. India is a mega-diversity nation due to the richness of a large variety of plants and animals. The plants had been used for the healing of diseases ages ago before the use of recent chemical drugs. *Murraya koenigii* is a versatile tree seen all over India, Srilanka, South East Asia and

belonging to family the Rutaceae commonly is known as *curry* leaf, meethi neem, *karipatta*, krishnanimba etc. (Balakrishnan *et al*., 2020). It is a small tree of about 6 meters in height and 14 to 42 cm in diameter and is cultivated throughout India for its aromatic leaves. The various phyto-constituents are described by different researchers from different localies showed different pharmacological activities (Saini *et al*., 2013; Sujith *et al*., 2018). Its leaves are used as an analgesic, anti-inflammatory, anthelmintic, antimicrobial, antifungal, antiprotozoal, antioxidant, hepatoprotective, nephroprotective, cytotoxic, mosquitocidal and curing piles, itching, dysentery, diarrhea, vomiting, leucoderma, blood disorders etc. (Saini and Reddy, 2015; Goel *et al*., 2020). The important phyto-constituents have been isolated and characterized *viz*., alkaloids (mahanine, koenine, koenimbine, Isomahanine), coumarin glycoside (scopotin, murrayanine, carotene, oxalic acid), essential oil (Di-alpha phellandrene, D-terpinol, caryophyllene), lutein, tocopherol, murrayanol etc.

Eucalyptus globulus is a large evergreen tree belonging to the genera of Myrtaceae family. It is commonly known as blue gum, safeeda, forest red gum is purely a native species of Australia and Tasmania, but now has been cultivated in India and Tunisia for its use in paper production alongside pharmaceutical and cosmetics. The leaf extracts have been known traditionally to the heal wound and to possess stimulant, carminative, antibacterial, expectorant, antifungal, anti-inflammatory, antiseptics, febrifuge, antioxidant, mosquito repellent and keen anthelmintic properties (Sastya *et al*., 2018; Kaur *et al*., 2019). Although, various volatile phyto-chemicals like isoprenoids found in the leaves showed antimicrobial properties. Therefore, the present investigation is to identify the preliminary phyto-chemical constituents of *M. koenigii* and *E. globulus* leaves extracts.

(A) *E. globulus*

(B) *M. koenigii*

Fig. 1. Natural shade drying of freshly collected leaves materials.

MATERIALS AND METHODS

A. Duration and place of work

The proposed study was carried out in Department of Veterinary Parasitology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur (Madhya Pradesh), during the year 2020-21. The Jabalpur city is located in Mahakoshal region of central part of India and of latitude 23^{o} 10' N and longitude 79^{o} 56' E with an average height of 411 m above mean sea level.

B. Collection and processing of plants material

Fresh leaves of curry (*M. koenigii*) and eucalyptus (*E. globulus*) were collected from the Veterinary College Campus, Jabalpur (Madhya Pradesh). The plants materials were identified and authenticated by the botanist of the Department of Botany, Jawahar Lal Nehru Krishi Vishwa Vidhyalaya, Jabalpur. The leaves of both plants were cleaned manually and kept under shade at a well-ventilated place in the laboratory till the total absence of moisture content from the leaves (Fig 1). Furthermore, the plant material was pulverized to powder form with a mixer grinder and stored in an airtight container.

C. Preparation of the plants extracts

The aqueous and methanolic extracts were prepared from the leaves of the above mentioned plants. For aqueous and methanolic extract, 50 gram of powdered sample of each plants material was soaked in 400 ml of distilled water and analytical grade methanol (covered with aluminum foil), respectively in a glass flask and stirred at hourly intervals initially 3-4 times and then, left undisturbed (8 h for aqueous extract) for soaking at room temperature and then filtered through Whatman filter paper No. 1 with separating funnel. Soaking for methanolic extract was done for a period of 72 h. Finally obtained filtrate was concentrated by evaporation at 30-35^{o}C under a biological incubator with some modification in procedure described by Bendigeri, (2019). The extracts were kept in air tight containers and marked individually and preserved at 4^{o}C in the refrigerator till their further use (Fig. 2). The percentage yield of each extracts was calculated as per Kanojiya *et al.* (2015).

$$\text{Extraction yield (\%)} = \frac{(w_1 \times 100)}{w_2}$$

Where, w_1 = Weight of extract obtained after evaporation the solvent

w_2 = Weight of the plants powder used

D. Phytochemical screening

The screening for different phyto-constituents of both aqueous and methanolic was carried out by using the standard protocol mentioned by Bendigeri *et al.* (2019).

(i) Test for alkaloids. For the detection of alkaloids, extracts (0.5 to 0.6 g) were mixed in 8 ml of 1% HCl, warmed and 2 ml of filtrate extract were treated separately with the following reagents.

Dragendorff's reagent – Firstly, 2 ml of filtrate was added to Dragendorff's reagent and the development of turbidity of precipitation was considered as the presence of alkaloids.

Wagner's reagent - The remaining 2 ml filtrate was added to Wagner's reagent and the development of brown flocculent precipitate indicated the presence of alkaloids.

(ii) Test for tannins. The tannins were detected by gradually adding methanol to the residue of the extracts. The solution was heated gently and obtained filtrates were treated with different reagents.

Lead acetate test- Firstly 2-3 drops of lead acetate solution were added to the above mentioned extract solution. The formation of precipitate suggested the presence of tannins.

Ferric chloride test- Few drop of ferric chloride solution were added to the above filtrate. The presence of tannins is confirmed by a greenish coloration in the filtrate of the extracts.

(iii) Test for saponins (Foam test). 1 ml of extracts was taken in a test tube and a small amount of sodium bicarbonate and water were added and shaken vigorously. The formation of froth showed the presence of saponins.

(iv) Test for sterols (Sulkowski test). 1 gram residue of the extract was taken in 2 ml chloroform followed by adding of 2 ml concentrated sulphuric acid by the side of the tube. The tube was gently shaken for a few minutes followed by the development of red color in chloroform layer and greenish yellow fluorescence in lower layer confirmed presence of sterol in the extract.

(v) Test for fixed oils (Filter paper strip test). A small drop of extracts was put on filter paper. Appearance of oil spot indicated positive test for the presence of fixed oils.

(vi) Test for proteins (Biuret test). For the detection of proteins, 1 g of residue of the extract was taken in water and 1 ml of 4% NaOH solution was added. The violet pink color confirmed the presence of proteins.

(vii) Test for Anthraquinones (Bontrager's test). A small amount of extract boiled for a few minutes with 5 ml of 10% sulphuric acid and filtered immediately while hot then the filtrate was cooled and shaken with benzene. The benzene layer was separated and shaken with half of its volume of 10% ammonia. The ammonical layer acquiring pink color indicated the presence of anthraquinones.

(viii) Test for flavonoids. 1 ml of extract was dissolved in 5 ml of 95% ethanol and a few drops of diluted NaOH solution were added. The intense yellow color appeared in the test tube. It became colorless with the addition of a few drops of diluted Hcl showed the presence of flavonoids.

(ix) Test for reducing sugars. Initially 5 ml of different extract solution was poured in a test tube followed by the addition of an equal quantity of Benedict's reagent and heated subsequently. The result is shown by the appearance of brown red precipitate indicated presence of reducing sugars.

(x) Test for glycosides. Test for glycosides are followed by reducing sugars. The solution obtained in Benedict's test was filtered and diluted HCl was added slowly. An equal quantity of Benedict's reagent was added and boiled frequently. The test is judged by the appearance of brownish precipitate revealed the presence of glycosides.

(xi) Test for resins. Resins are detected by taking a little amount of extract residue dissolved in alcohol and adding a few drops of distilled water. The appearance of turbidity was considered as an optimistic test for resin.

RESULTS AND DISCUSSION

A. Extraction yield and physical properties of plant extracts

The highest yield of aqueous and methanolic *E. globulus* leaves extracts were 13.07% and 17.17%, respectively whereas for *M. koenigii* leaves extracts were 09.02% and 16.71%, respectively. However, our findings are contrary inconsistent with the findings of Kanojiya *et al.* (2015) where they reported that methanolic extract has a lower yield (11.84 %) as compared to aqueous (13.30 %) eucalyptus leaves extract. The black colored extract was observed in aqueous eucalyptus leaves, whereas a greenish brown extract was observed in the case of methanolic eucalyptus leaves extracts (Fig. 2). Yadav *et al.* (2010) revealed the highest yield (28.42%) with yellowish brown color, non sticky nature and peculiar odor in crude methanolic extract of eucalyptus leaves. The brownish black and brown colors were observed in aqueous and methanolic curry leaves extracts, respectively. The solid or powdery consistency was seen in aqueous eucalyptus leaves and methanolic curry leaves extracts. The solid consistency was seen in methanolic eucalyptus leaves extract while semi-solid in aqueous curry leaves extract. The color of curry leaves extracts are in partial agreement with the findings of Saini *et al.* (2013) they showed characteristic odor with methanolic yield (13.05%) are almost similar finding as revealed in our study.

(A) Aqueous (*E. globulus*)

(B) Methanolic (*M. koenigii*)

Fig. 2. Extracts of leaves materials.

B. Phyto-chemical analysis

M. koenigii aqueous leaves extract was found positive for alkaloids, tannins, saponins, flavonoids whereas methanolic extracts showed the presence of proteins, glycosides, tannins with ferric chloride test and flavonoids constituents are negligible finding (Table 1, Fig 3). Interestingly, alkaloids, tannins and saponins were detected for both aqueous and methanolic leaves extract with Dragendroff's, Wagner's, lead acetate and foam test, respectively. However, a study a Uraku and Nwankwo (2015) revealed high quantities of flavonoids, alkaloids, tannins while low levels of saponins and carotenoids in their study. Another characteristic finding in our study was a complete absence of sterols, fixed oils, anthraquinones as well as reducing sugars in curry leaves extract. Molla and Bandyopadhyay, (2016) detected major secondary metabolites such as tannins, saponins, phenols, triterpenoids, flavonoids in curry leaves methanolic extracts are as confirmatory findings except for flavonoids. Rashmi and Naveen (2016) reported alkaloids, carbohydrates, tannins, terpenoids in all prepared extracts (aqueous, methanolic, ethanolic), however cardiac glycosides and phylobatannins were identified only in ethanolic and methanolic extracts. Furthermore, the study by Tomar *et al.* (2017) revealed steroids in both aqueous and methanolic extracts of *M. koenigii* which are unique findings compared to our study. They also reported tannins and flavonoids are only components found in methanolic and aqueous leaves extract, respectively. Sujith *et al.* (2018) suggested similar findings in relevant to phyto-constituents *viz.*, alkaloids, glycosides, tannins, while saponins and proteins are unique findings in methanolic curry leaves extracts. Similar finding in curry leaves aqueous extracts except alkaloids, phenolics and terpenes when compared to above researcher in their study. The phyto-chemical constituents of both plants extracts were mentioned in Table 1. Kaloni *et al*. (2020) demonstrated alkaloids (murrayacinine, mahanine) are more potent phyto-chemicals than flavonoids and considered as anti-inflammatory compounds in the management of rheumatoid arthritis. Abuga *et al.* (2020) studied that ethyl acetate leaf extract of *M. koenigii* has broad spectrum antibacterial activity which could be due to the presence of phenolic compounds whereas Abeysinghe *et al.* (2021) evaluated antibacterial as well as an antioxidant activity because of total phenol and flavonoid contents in *M. koenigii* leaves extract.

Table 1: Phyto-chemical constituents of plants leave extracts.

Phyto-chemical parameter		**Leaves extract**			
		E. globulus		***M. koenigii***	
Active principle	**Phyto-chemical Tests**	**Aqueous**	**Methanolic**	**Aqueous**	**Methanolicc**
Alkaloids	Dragendroff's test	+	-	+	+
	Wagner's test	+	+	+	+
Tannins	Lead acetate test	+	+	+	+
	Ferric chloride test	-	-	+	-
Saponins	Foam test	-	+	+	+
Sterols	Sulkowski test	-	+	-	-
Fixed oils	Filter strip test	-	-	-	-
Proteins	Biuret test	-	+	-	+
Anthraquinones	Bontragers's test	-	+	-	-
Flavonoids	-	-	-	+	-
Glycosides	Benedict's test	+	+	-	+
Reducing sugar	Benedict's test	+	-	-	-

(Where, '+' indicate positive test, '-' indicate negative test)

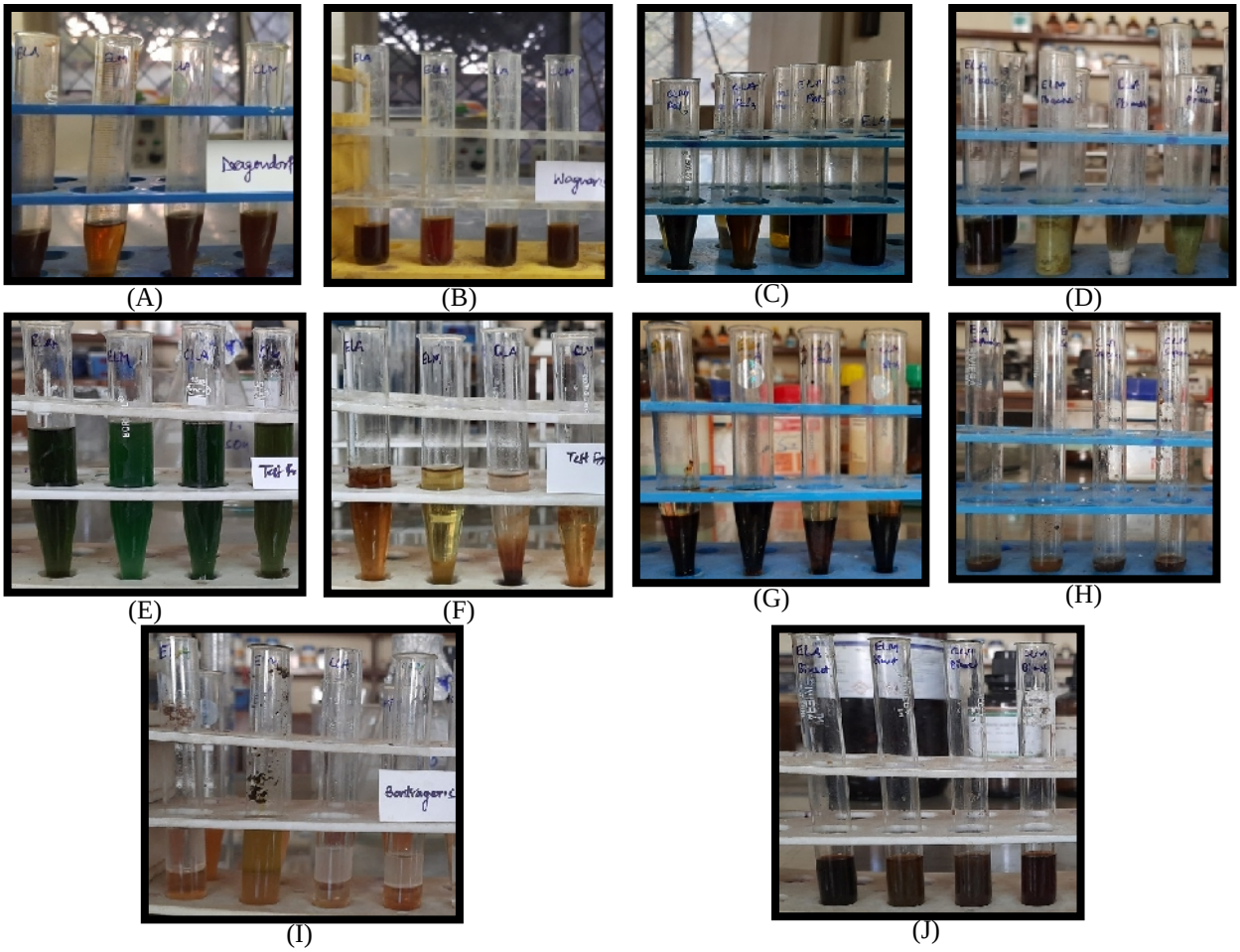

Fig. 3. Phyto-chemistry of plants extracts.
Tests for alkaloids: (A) Dragondorff's test (B) Wagner's test
Tests for tannins: (C) Ferric chloride test (D) Lead acetate test
(E) Benedict's test (F) Test for flavonoids (G) Sulkowski test (H) Foam test (I) Bontrager's test (J) Biuret test

E. globulus aqueous leaves extract was found to be positive for alkaloids, tannins (lead acetate test), carbohydrates, glycosides and reducing sugars while methanolic extract showed the presence of alkaloids (Wagner's test), tannins (lead acetate test), glycosides and saponins, sterols, proteins, anthraquinones are the additional findings (Table 1, Fig. 3). While, comparison to both types of extracts (aqueous and methanolic), Wagner's test, lead acetate test, Benedict's test were tested positive for alkaloids, tannins and glycosides, respectively. However, tannins, flavonoids and steroids in crude aqueous and methanolic extracts have been observed by Sharma *et al.* (2009) while Bhagat *et al.* (2012) suggested anthraquinones, saponins, tannins, flavonoids and cardiac glycosides in methanolic extract. Furthermore study by Javed *et al*. (2012) showed partially agreement where they reported alkaloids, tannins, glycosides in aqueous and in addition to steroids and phenols in methanolic extract. Indistinguishable finding was reported by Kanojiya *et al.* (2015) where they reported sterols are unique findings in aqueous leaves extract which could be due to using of different extraction methods. The methanolic extract observed positive for flavonoids and fats in addition to the alkaloids, carbohydrates, steroids and tannins in context to our finding where there is a lack of flavonoids and fats. Remini *et al.* (2016) demonstrated the antioxidant effect in the crude extract of *E. globulus* fruits is higher than that of its leaves due to the presence of a higher level of total phenolics and tannins. However, Sastya *et al.* (2018) revealed inconsistent findings with respect to flavonoids, saponins and triterpenes in aqueous eucalyptus leaves extract whereas glycosides are additional finding in our study. However, in methanolic extract they reported reducing sugar and triterpenes which are totally absent along the presence of additional phyto-constituents like sterols, proteins and anthraquinones in our study. Thus, the present illustrated table 1 enlightened the maximum numbers of phyto-chemicals retained in the methanolic eucalyptus leaves extract than that of the aqueous extract. Data revealed in our study showed lack of flavonoids from aqueous and methanolic eucalyptus leaves extracts are comparable to the above researcher finding. Our findings are in agreement with the finding of Kaur *et al.* (2019) regarding constituents, such as

carbohydrates, saponins, tannins whereas alkaloids, sterols, proteins, anthraquinones are the additional findings in our study. Furthermore, the study by Ajilore *et al.* (2021) is in partial agreement with our finding except for flavonoids whereas phlobatannins and terpenoids are additional constituents.

CONCLUSION

The highest yield for both aqueous and methanolic extract was observed in leaves of *E. globulus* as compared to that of *M. koenigii* leaves extracts. Black colored aqueous and greenish colored methanolic extracts for *E. globules* leaves and brownish black aqueous and brown colored methanolic extract were observed for *M. koenigii* leaves. The solid or powdery consistency was observed in aqueous eucalyptus and methanolic curry leaves extracts. Solid and semi-solid consistencies were noted in methanolic eucalyptus leaves extract and aqueous curry leaves extract, respectively. The aqueous and methanolic *M. koenigii* leaves extract were found to possess alkaloids, tannins, saponins and flavonoids whereas proteins were additional constituents in the methanolic extract. The aqueous and methanolic *E. globulus* leaves extracts were found to possess alkaloids, tannins and glycosides whereas saponins, sterols, proteins, and anthraquinones were the additional constituents in the methanolic extracts. Further investigations need to be carried out for identification and quantification of phytochemicals in these medicinal plants and their judicious use in the treatment of various animal and human infections.

FUTURE SCOPE

Environmental safety is of primary considered while using the plants extracts. Thus, these drugs are not related to any type of environmental persistent and without resistance as well as residue in the processed product. With the help of sophisticated technique the drugs must be assessed properly before release into the market. Therefore, there is need of collaborative research by comparing the efficacy of different plants material against pathogenic entities to evaluate their potency or efficacies. Hence we have to know about the content of any plant material in respect of any challenges and constraints.

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Conflict of Interest. None.

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