

RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF AERIAL PARTS OF *CROTALARIA PALLIDA*Vinuta Mallappa Hittalamani*¹, U. Sunil Chandra², N.B. Shridhar³, B. Kavitha Rani⁴, D. Niranjan⁵ and M.S. Rudraswamy⁶

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Abstract: The genus *Crotalaria* belongs to the family Fabaceae which is the third largest genus of this family, containing approximately six hundred species widely distributed in tropical and subtropical regions of the world. The annual erect herb *Crotalaria pallida* Aiton is referred to as "rattlesnake," *C. pallida* is frequently used in traditional medicine to treat a wide range of ailments, including diarrhoea, diabetes, skin infections, snake bites, and urinary issues. An experimental study was undertaken to assess the phytochemical constituents of *C. pallida* by using methanol as solvent for the extraction of aerial parts of the plant. The sieved plant powder was soaked in methanol at 1:5 ratio in glass containers for one week and then filtered and subjected to extraction using rotary evaporator (hot extraction method). Finally obtained gummy extract was used for phytochemical screening tests. The distilled water was used as solvent for extract for some of the tests. All the tests were performed as per standard protocol using the chemicals and reagents procured from authenticated chemical manufacturers and results were recorded. The Physical characteristics, per cent yield were also estimated in which per cent yield was 3.702%, dark brownish green colour and gummy-sticky consistency of extract were also found. The phytochemical screening of *C. pallida* revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, phytosterols and triterpenoids, while the extract was negative for the presence of carbohydrate, amino acids and protein. The phytoconstituents of the plant might play an important role in therapeutic and toxic properties.

Keywords: *Crotalaria pallida*, Phytochemical, Therapeutic, Toxic, Methanolic extract, Aerial parts

INTRODUCTION

In India, the genus *Crotalaria* consists of eighty-one species, of which twenty-seven are endemic and fifteen are listed in the Red Data Book of Indian Plants. Some species of *Crotalaria* are grown for ornamental purposes. This genus is generally self-adapted to tropical climates, with few members found in temperate regions (Samba *et al.*, 2002) Many species become toxic due to the accumulation of pyrrolizidine alkaloids during flowering and sowing (Nuhu *et al.*, 2002). Nevertheless, *Crotalaria* species play an important role in veterinary medicine and treatment of many diseases (Nuhu, 1999). *C. pallida* is found to be across the various geographical regions of India like, western ghats of Karnataka, Gujarat, Maharashtra, Goa, and Kerala; also, in the states of Sikkim, Tamil Nadu (Ramachandran *et al.*, 1988; Sanjappa, M.

1991). It is also distributed in Assam, Bihar and Odisha (Barooah and Ahmed, 2014).

Episodes of toxicities suspected to be due to consumption of the plant *C. pallida* and deaths among the sheep have been noticed by the veterinarians and farmers from Hassan and Shivamogga districts of Karnataka state over the last five years. As per the information received, the sheep had exhibited the clinical signs such as the sudden onset of bloat, hematuria, melena, and recumbence before death. On reviewing the literature regarding the properties of *C. pallida*, revealed that the parts of the plant had a therapeutic value with several medicinal and ethnobotanical applications as per several folklore claims of medicinal properties of the aerial parts of the plant. However, there was scarcity of the data on the nature and type of toxicities resulting from *C. pallida*.

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PLATE-1

Crotalaria pallida plant and its parts

A. Plant



B. Leaf – trifoliate –dorsal



C. Leaf – trifoliate -ventral



D. Flowers



E. Pods



F. Dried pod and seeds

MATERIALS AND METHODS

The fresh aerial parts of the plant were collected in the month of May and June 2022 in and around Gajanur village, Shivamoggataluk, Shivamogga District, Karnataka State and Veterinary College Shivamogga campus. The taxonomic identification of the plant was certified by Dr. Rajeshwari. N, Professor, Department of Botany and seed technology, Sahyadri Science College, Shivamogga,

verified the plant's taxonomic identification. The plant material was collected, cleaned under running water, and then dried in the shade for about three weeks. The plant material was mechanically ground and a coarse powder was obtained and sieved into a fine powder, which was then stored in airtight containers until further phytochemical study.

Chemicals

The chemicals employed in the current investigation were commercially purchased from authenticated

dealers. Absolute ethanol, acetone, 10% anhydrous ferric chloride (An. FeCl₃), concentrated nitric acid (Conc. HNO₃), copper turnings, methanol, picric acid and 10% 1N Sodium hydroxide (NaOH) were procured from Sd Fine Chem. Ltd., Mumbai.

Chloroform, ether and 10% sodium chloride (NaCl) were procured from RFCL Ltd., New Dehli. 10% ammonia solution (NH₄OH), bromine water and concentrated sulphuric acid (Conc. H₂SO₄) by Nice Chemicals Pvt. Ltd, Cochin. α -naphthol, 0.1% ascorbic acid, concentrated 1M Hydrochloric acid (Conc. HCl), copper sulphate (CuSO₄), disodium hydrogen phosphate, DPPH (1,1-diphenyl-2-picrylhydrazyl), 1% gelatine, 1% glacial acetic acid, iodine, magnesium ribbon, mercuric chloride, 10% lead acetate, 1% potassium ferricyanide, sodium nitroprusside, sodium bi-carbonate (NaHCO₃), sulphanic acid and Fehling's reagents 1 & 2 from (Hi-media Laboratories Pvt. Ltd.). Ninhydrin, potassium Iodide (KI), potassium chloride (KCl), potassium dihydrogen phosphate, 10% trichloro acetic acid from Merck Pvt. Ltd.

Reagents

Commercially available reagents purchased from Nice Chemicals (P) Ltd. included Barfoed's reagent, Benedict's reagent, Biuret reagent, Dragendorff's reagent, Hager's reagent, Mayer's reagent, Millon's reagent, Molisch's reagent, Ninhydrin reagent, Robert's reagent, Seliwanoff's reagent and Wagner's reagent.

Preparation of the extract

Preparation of plant extract for the experimental study is the first step, before moving further with the desired biological testing as, it entails the extraction and assessment of the quality and amount of bioactive elements. The type of plant, the portion of the plant to be extracted, the makeup of the bioactive chemicals, and availability of solvent would have impact on the solvent of choice (Abubakar and Haque, 2020). Methanol was preferred as the solvent for preparing the MECP.

Soaking

In the ratio of 1:5 (powder/solvent) was used to soak 1000 g of the coarse powder in 5 L of methanol (99% Sd fine Chem.® Ltd) in closed glass flasks at room temperature. For the first six hours, the soaked mixture was stirred with an electric orbital shaker (REMI®) to ensure that the solvent and powder were properly mixed. Later for 5 days, flasks were shaken and stirred three times daily (Odey *et al.*, 2012).

Filtration and concentration

After one week, the contents were initially filtered through muslin cloth, then with Buchner's funnel and Whatman No. 1 filter paper (Hi-media® Laboratories Pvt. Ltd, 24 cm). As the solvent evaporated and the extract settled, the filtrates were then individually concentrated in a vacuum using a rotary evaporator (DLAB® RE100-Pro) at 40 °C with 92 rpm for 1 hour. These were concentrated and dried completely in the SLM-INC-OS-250 incubator at 40

°C for 1 day. The extracts were kept in a refrigerator in airtight containers, and aliquots were taken for the production of the pharmacological preparation and phytochemical screening (Hasan *et al.*, 2013).

Calculation of yield

The *percent yield* (dry weight of extract) of MECP (w/w) was calculated using the formula as mentioned below

$$\% \text{ yield} = \frac{\text{Final weight of the extract}}{\text{Initial weight of the powder}} \times 100$$

Phytochemical screening

The tests for the presence of various phytoconstituents, such as alkaloids, flavonoids, saponins, carbohydrates, sterols and terpenoids, anthraquinone, glycosides, coumarins, tannins and phenolic compounds, were performed on MECP.

Qualitative phytochemical analysis

Organic analysis of primary metabolites

The MECP was subjected for qualitative phytochemical analyses using the tests and techniques as described by previous authors for the presence or absence of various phytoconstituents (Harborne, 1998; Raaman, 2006; Tiwari *et al.*, 2011; Hasan *et al.*, 2013; Banu and Cathrine, 2015; Silva *et al.*, 2017; Ramamurthy and Sathiyadevi, 2017; Balamurugan *et al.*, 2019).

The 100 mg of MECP was dissolved in 5 mL of distilled water and filtered. The filtrate was subjected for the following tests to detect the presence or absence of various phytoconstituents.

Test for carbohydrates

a. Barfoed's test (for monosaccharides)

In a test tube, 1 mL of the extract filtrate was taken and 1 mL of Barfoed's reagent was added and heated on a water bath for 2 min and observed for colour change.

b. Molisch's test

The filtrate of 2 mL was taken in a test tube and 2 drops of Molisch's reagent was added, the mixture was shaken well and 1 mL of conc. H₂SO₄ was added slowly along the sides of the test tube and allowed to stand.

c. Seliwanoff's test (for Ketoses)

Seliwanoff's reagent of 3 mL was added to 1 mL of the extract filtrate solution in a test tube and heated on a water bath for 1 minute and observed.

d. Benedict's test (for reducing sugar)

In a test tube about 0.5 mL of the filtrate was taken to which 0.5 mL of Benedict's reagent was added. This mixture was heated for about 2 minutes in a boiling water bath.

e. Fehling's reduction test

In a test tube 1 mL of filtrate and 1 mL each of Fehling's solution 1 and Fehling's solution 2 were added and boiled on water bath and observed for color change.

Test for starch

A test tube containing 5 mL of distilled water was added with the 10 mg of iodine, 75 mg of potassium iodide, and then 3 mL of the MECP was also mixed and shaken well.

Test for proteins

a. Biuret test

The extract filtrate of 2ml was treated with 1 drop of 2% copper sulphate solution and to this 1 mL of 95% ethanol was added, followed by excess of potassium hydroxide pellets.

b. Millon's test

The extract filtrate 2 mL was taken in a test tube and few drops of Millon's reagent were added and observed.

c. Xanthoproteic test

To the 1 mL of concentrated nitric acid, 2 mL of extract filtrate was added and then heated for 3 minutes. Then cooled and 0.5 mL of NaOH was added. If there is reddish orange color it indicates the presence of aromatic amino acids.

Test for amino acids

A test for amino acids was performed on the extract filtrate prepared by dissolving the 100 mg of extract in 10 mL of distilled water and filtering it through Whatman filter paper No. 1.

Ninhydrin test

The extract filtrate of 2mL was taken in a test tube and 2 drops of Ninhydrin reagent (10mg of ninhydrin in 200mL of acetone) were added and observed for color change.

b. Nitric acid test

The extract filtrate of 2 mL was taken in a test tube and few drops of nitric acid were added along the sides of the tube and observed.

Tests for gums and mucilage

Dissolve 100 mg of the extract was dissolved in 10 mL distilled water and 25 mL of absolute alcohol was added with constant stirring and observed.

Qualitative analysis of secondary metabolites

Test for alkaloids

The extract of 200 mg was dissolved in 5-6 mL of 1.5% HCl and filtered. The filtrate was tested with the alkaloid reagents.

a. Mayer's test

The filtrate of 200 mg was taken in a test tube and few drops of Mayer's reagent was added along the sides of test tube and observed for color change.

b. Wagner's test

The extract filtrate of 2 mL was taken in a test tube and few drops of Wagner's reagent were added by the sides of the test tube and observed for color change.

c. Hager's test

The extract filtrate of 2 mL was taken in a test tube and 2 mL of Hager's reagent was added and observed.

d. Dragendorff's test

The extract filtrate of 2 mL was taken in a test tube and 2 mL Dragendorff's reagent was added and observed.

Test for flavonoids

a. Aqueous sodium hydroxide test

After treating the extract with 10% NaOH solution, yellow color appears then few drops of concentrated hydrochloric acid was added and observed for color change.

b. Sulphuric acid test

A fraction of the extract was treated with few drops of concentrated sulphuric acid in a test tube.

c. Shinoda's test / Magnesium and Hydrochloric acid reduction test

The extract (50 mg) was dissolved in 5 mL of alcohol in a test tube and a few fragments of magnesium ribbon and concentrated hydrochloric acid (dropwise) were added and observed.

d. Lead acetate test

The extract filtrate of 1 mL was taken in a test tube and few drops of 10% of lead acetate were added and observed.

e. Alkaline reagent test

The extract filtrate of 2 mL was taken in a test tube and 2 mL of 10% ammonium hydroxide solution was added and heated and observed for color change.

Test for glycosides

The extract of about 0.5 g was hydrolyzed with 20 mL of 0.1 N hydrochloric acid and filtered using Whatman No.1 filter paper. The filtrate was used to test the presence of glycosides.

a. Borntrager's test

The extract filtrate of 2 mL was taken in a test tube and 3 mL of chloroform was added and shaken. The chloroform layer was separated and 10% ammonia solution was added.

b. Keller-Killiani test

The extract filtrate of 1 mL was taken in a test tube to which 1.5 mL of glacial acetic acid was added along the sides of the tube, one drop of 5% FeCl₃ and few drops of conc. H₂SO₄ were added.

c. Legal's test

With a few drops of 10% NaOH solution, concentrated extract was made alkaline. Then, freshly made sodium nitroprusside solution was added to the solution.

Test for phenolic compounds

a. Lead acetate

A small amount of extract was dissolved in 5 mL distilled water to which 3 mL of 10% lead acetate solution was added.

b. Gelatin test

A small amount of extract was dissolved in 5 mL distilled water to which 1% gelatin solution and 10% NaCl were added.

Test for tannins

a. Ferric chloride test

The extract filtrate of 50 mg was dissolved in 5 mL of distilled water to which few drops of neutral 5% ferric chloride solution were added and observed.

b. Bromine water test

The 500 mg of the plant extract was sprinkled into 5 mL of bromine water in a test tube, and the results were monitored.

Test for saponins

a. Froth test

The extract filtrate was diluted with distilled water to 20 mL. The solution was gently stirred in a graduated cylinder for 15 minutes. The presence of saponins is indicated by a 2 cm layer of foam.

Test for phytosterols and triterpenoids

The extract (0.5 g) was treated with 10 mL chloroform and filtered. The filtrate was used to test the presence of phytosterols and triterpenoids.

a. Leibermann-Burchard test

The extract filtrate of about 1 mL was taken in a test tube and 2 mL of acetic anhydride was added and to which 1 or 2 drops of concentrated H_2SO_4 was added along the sides of the test tube and observed.

b. Salkowski's test

The extract filtrate of 2 mL was taken in a test tube and a few drops of conc. H_2SO_4 was added, shaken, and allowed to stand.

Test for fixed oils and fats

a. Oily spot test

A little amount of extract is placed between two filter papers and then pressed gently. Oily stain indicates the presence of fixed oils.

RESULTS

Phytochemical analysis

Physical nature and pH of the extract

Physical nature and percentage yield of MECP is presented in table 1. The percentage yield and pH of MECP was 3.702% and 4.51 respectively.

Table 1. Physical nature and *per cent* yield of MECP

Sl. No.	Details	Results
1	Solvent used	Methanol
2	Extract colour	Dark brownish green
3	Consistency	Gummy - sticky
4	Per cent Yield (w/w)	3.702 %
5	pH	4.51

Organic analysis

The MECP was subjected to preliminary phytochemical analysis and the results were illustrated in table 9 and Plate 4, 5, 6, and 7.

Qualitative analysis of primary metabolites

Test for carbohydrates

a. Benedict's test

Absence of the formation of characteristic red precipitate with the test revealed the absence of sugars in MECP.

b. Molisch's test

There was no formation of violet colour ring at the junction of the liquids indicated the absence of carbohydrates in MECP.

c. Fehling's reduction test

Negative for formation of brick red colour precipitate was observed which indicated the absence of carbohydrates in MECP.

d. Barfoed's test

There was no formation of red precipitate indicated absence of carbohydrates (monosaccharides) in MECP.

e. Seliwanoff's test

The non appearance of rose red color indicated that the result was negative for the presence of carbohydrate in MECP.

Test for starch

MECP was negative for starch as indicated by the lack of development of blue colour.

Test for proteins

a. Biuret test

The test was negative as indicated by lack of formation of pink/violet colour in ethanolic layer indicating the absence of protein in MECP.

b. Millon's test

The test was negative for presence of proteins in MECP as evident by absence of formation of white precipitate.

c. Xanthoproteic test

The absence of brownish-yellow or reddish orange colour revealed that the MECP was negative for proteins.

Test for amino acids

a. Ninhydrin test

The MECP was negative for amino acids as indicated by absence of blue or violet colour.

b. Nitric acid test

The failure of appearance of yellow color indicated the absence of protein and free amino acids in MECP.

Test for gums and mucilage

The absence of white or cloudy precipitate, indicated the absence of gums or mucilage in MECP.

Qualitative analysis of secondary metabolites

Test for alkaloids

a. Mayer's test

Development of cream coloured precipitate indicated that MECP was positive for the presence of alkaloids.

b. Wagner's test

The reddish brown coloured precipitate observed indicated the presence of alkaloids in MECP.

c. Hager's test

The formation of yellow precipitate indicated that the test was positive for the presence of, alkaloids in MECP.

d. Dragendroff's test

The formation of reddish-brown precipitate indicated that, MECP was positive for the presence of alkaloids.

Test for flavonoids

a. Aqueous sodium hydroxide test (AqNaOH test)

The development of a strong fluorescent yellow colour a, which later turned colourless, confirmed the presence of flavonoids in MECP.

b. Sulphuric acid test

The development of orange coloured precipitate indicated the presence of flavonoids in MECP.

c. Magnesium and Hydrochloric acid reduction test (Mg-HCl reduction test)

Development of crimson colour, indicated the presence of flavonoids in MECP.

c. Lead acetate test

Formation of yellow precipitate was positive inference for the presence of flavonoids.

d. Alkaline reagent test

Flavonoid's presence was detected in MECP as the development of intense fluorescence yellow colour.

Test for glycosides

a. Borntrager's test

Lack of formation of deep pink colour indicated the glycosides were absent in the MECP.

b. Keller-Killani test

At the interphase of the two aqueous layers, a reddish-brown colour developed, which indicated the presence of glycosides in MECP.

c. Legal's test

There was lack of development of blue colour, which confirmed the absence of glycosides in MECP.

Test for phenolic compounds

a. Lead acetate test

The formation of white coloured precipitate indicated presence of phenolic compounds in the MECP.

b. Gelatin test

The development of bulky white precipitate showed that MECP was positive for presence of phenol.

Test for tannins

a. Ferric chloride test

The development of a dark green colour was seen, which indicated the presence of tannins in MECP.

b. Bromine water test

The presence of tannins in MECP was confirmed by the decolorization appearance of bromine water.

Test for saponins

a. Froth test

The development of a persistent froth that remained relatively stable for five minutes made it evident that MECP was positive for presence of saponins.

Test for phytosterols and triterpenoids

a. Lieberman-Bucharat test

At the intersection of two layers, a deep red ring was observed, which indicated the presence of triterpenes in MECP.

b. Salkowski test

The lower layer turning reddish brown in appearance revealed the presence of sterols in the MECP.

Test for fixed oils and fats

a. Oily spot test

The absence of oily stain on filter paper indicated that the MECP was negative for presence of fixed oil and fats.

Inference

Preliminary phytochemical analysis of MECP revealed that, the extract revealed the presence of phytoconstituents viz.: alkaloids, flavonoids, phenolic compounds, tannins, phytosterols and triterpenoids. The results of phytochemical tests are as presented in table 2 and Plate 2, 3, 4 and 5.

Table 2. Preliminary phytochemical analysis of MECP.

Sl No	Tests	Result
Primary metabolites		
Carbohydrates		
1	a. Benedict's test	- ve
	b. Molisch's test	- ve
	c. Fehling's test	- ve
	d. Barfoed's test	- ve
	e. Seliwanoff's test	- ve
2	Test for Starch	-ve
Proteins		
3	a. Biuret test	- ve
	b. Millon's test	- ve
	c. Xanthoproteic	- ve

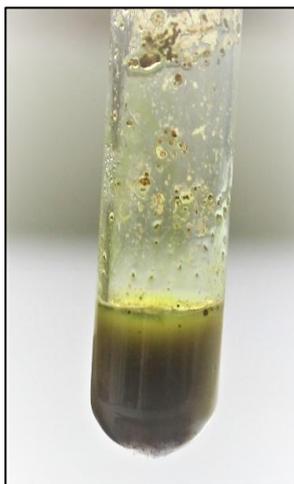
4	Amino acids	
	a.Ninhydrintest	- ve
	b.Nitricacidtest	- ve
5	Test for gumsandmucilage	- ve
Secondary metabolites		
1	Alkaloids	
	a.Mayer'stest	+ ve
	b.Wagner'stest	+ ve
	c.Hager'stest	+ ve
	d.Dragendroff'stest	+ ve
2	Flavonoids	
	a.Sulphuricacidtest	+ ve
	b. Aqueous Sodium hydroxide test	+ ve
	c.Magnesium and Hydrochloricacid reduction test	+ ve
	d.Leadacetatetest	+ ve
	e.Alkaline reagenttest	+ ve
3	Glycosides	
	a.Bomtrager'stest	- ve
	b.Keller-Killian itest	+ ve
	c.Legal'stest	- ve
4	Phenolic compounds	
	a.Leadacetatetest	+ ve
	b.Gelatintest	+ ve
	c.Ferricchloridetest	+ ve
5	Tannins	
	a. Gelatintest	+ ve
6	Saponins	
	a.Frothtest	+ ve
7	Phytosterols and triterpenoids	
	a.Leiberman-Bucharatetest	+ve
	b.Salkowaskitest	+ ve
8	Fixed oils and fats	
	a. Oily spot test	- ve

+ve :Positive

-ve :Negative

PLATE 4

Results of the phytochemical analytical tests of MECP



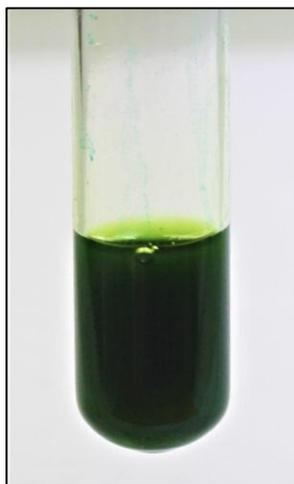
A. Benedict's test: - ve



B. Molisch's test: - ve



C. Fehling's test: - ve



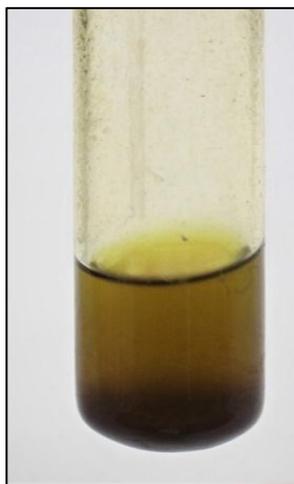
D. Barfoed's test: - ve



E. Seliwanoff's test: -ve



F. Test for starch: - ve



G. Biuret test: -ve



H. Millon's test: - ve



I. Xanthoproteic test: -ve

PLATE 5

Results of the phytochemical analytical tests of MECP



A. Ninhydrin test: - ve



B. Nitric acid test: - ve



C. Test for gums & Mucilages: -ve



D. Mayer's test: + ve



E. Wagner's test: + ve



F. Hager's test: + ve



G. Dragendorff's test: +ve



H. AqNaOH test: +ve



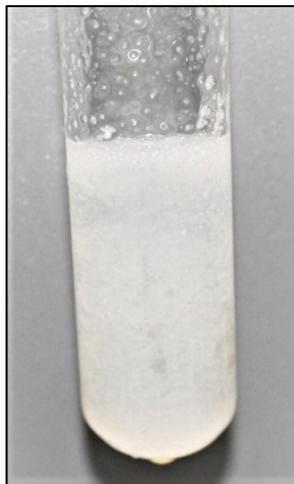
I. H₂SO₄ test: +ve

PLATE 6

Results of the phytochemical analytical tests of MECP



A. Mg-HCl reduction test: +ve



B. Lead acetate test: +ve



C. Alkaline reagent test: +ve



D. Borntrager's test: -ve



E. Keller-Killiani test: +ve



F. Legal's test: -ve



G. Lead acetate test: +ve



H. Gelatine test: +ve

I. FeCl₃ test: +ve

PLATE 7

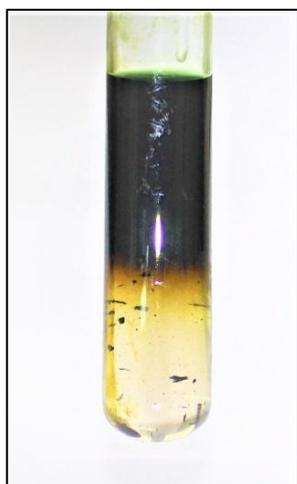
Results of the phytochemical analytical tests of MECP



A. Bromine water test: + ve



B. Froth test: +ve



C. Lieberman-Bucharat test: + ve



D. Salkowski test: +ve



E. Oily spot test: -ve

DISCUSSION

Crotalaria pallida contained various phytochemical constituents with therapeutic properties and has been previously reported to be used in traditional medicine

to treat various ailments due to various therapeutic effects.

As per Cowan (1999), methanol was the preferred solvent to obtain biologically active compounds like anthocyanins, flavones, lactones, phenones,

polyphenols, saponins, terpenoids, tannins, and xanthoxylines. The aerial parts of *Crotalaria pallida* were subjected for methanolic extraction, with the resultant extract being evaluated for phytochemical analysis, the antipyretic and *in vitro* antioxidant properties, before assessing the safety with repeated doses of the extract for 28 days in rats by analyzing haematological, biochemical parameters and the histopathological observation of organs at term.

The physical nature of methanolic extract of the aerial parts of *C. pallida* (MECP) was dark brownish green colour with gummy-sticky consistency. A lower per cent yield of 3.702 % w/w was estimated in the present study. The pH of the extract was 4.51. The colour, consistency and the per cent yield of the methanolic extract was in agreement with Bulbul *et al.* (2017), who have reported similar dark brown colour with gummy consistency for the ethanolic extract of *C. pallida*.

The phytochemical analysis of MECP revealed the presence of alkaloids, flavonoids, saponins, phenolic compounds, tannins, steroid and terpenoids. The MECP was negative for the presence of carbohydrates, starch, proteins and amino acids. The findings of phytochemical analysis of *C. pallida* in the present study were in agreement with the previous reports of Govindappa *et al.* (2011a & b) and Panda *et al.* (2015a), who had reported the presence of similar phytoconstituents, although in distilled water, ethyl acetate, ethanol, n-butanol, chloroform and petroleum ether extracts.

CONCLUSION

The physical nature of the MECP revealed the acidic pH, gummy-sticky consistency after extracting with the solvent methanol. The preliminary phytochemical analysis of MECP revealed the presence of various phytoconstituents like alkaloids, flavonoids, phenolic compounds, tannins, saponins, phytosterols and triterpenoids, while the extract was negative for the presence of carbohydrates, amino acids and protein. The phytoconstituents of the plant which might play an important role in therapeutic and toxic properties need to be identified for further exploration of the active toxic principle.

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REFERENCES

Abubakar, A. R. and Haque, M. (2020). Preparation of medicinal plants: Basic extraction and

fractionation procedures for experimental purposes. *J. Pharm. Bioallied Sci.*, **12** (1): 1-10.

[Google Scholar](#)

Balamurugan, V., Fatima, S. and Velurajan, S. (2019). A guide to phytochemical analysis. *Int. J. Adv. Res. Innov. Ideas Educ.*, **5**(1):236-245.

[Google Scholar](#)

Banu, K.S. and Cathrine, L. (2015). General techniques involved in phytochemical analysis. *Int. J. Adv. Res. Chem. Sci.*, **2**(4):25-32.

[Google Scholar](#)

Barooah, C. and Ahmed, I. (2014). Plant Diversity of Assam (A checklist of Angiosperms and Gymnosperms). Edn. 1st., Assam Science Technical and Environmental Council, BigyanBhawan, Guwahati, Assam, pp. 10-27.

[Google Scholar](#)

Bulbul, I.J., Fashiuddin, S. B., Haque, M. R., Sultan, M. Z. and Rashid, M. A. (2017). Antinociceptive and anti-inflammatory activities of *Crotalaria pallida* Aiton (Fam: Fabaceae) leaves. *Bangladesh Pharm. J.*, **20** (2): 165-171.

[Google Scholar](#)

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clin. Mic. Rev.*, **12** (4): 564-582.

[Google Scholar](#)

Flores, A. S., Filliettaz, A. M. and Tozzi, A. M. G. D.A. (2006). Taxonomic novelties in Brazilian species of *Crotalaria* Sect. *Calycinae* Wight & Arn. (Leguminosae-Papilionoideae). *Rodriguésia*, **57**: 127-130.

[Google Scholar](#)

Govindappa, M., Bharath, N., Shruthi, H. B. and Santoyo, G. (2011b). *In vitro* antioxidant activity and phytochemical screening of endophytic extracts of *Crotalaria pallida*. *Free Radic. Antioxid.*, **1** (3): 79-86.

[Google Scholar](#)

Govindappa, M., Bharath, N., Shruthi, H. B., Sadananda, T.S. and Sharanappa, P. (2011a). Antimicrobial, antioxidant and *in vitro* anti-inflammatory activity and phytochemical screening of *Crotalaria pallida* Aiton. *Afr. J. Pharm. Pharmacol.*, **5**(21): 2359-2371.

[Google Scholar](#)

Harborne, A. J. (1998). Phytochemical methods: A guide to modern techniques of plant analysis. Edn. 3rd., Chapman and Hall, London, pp. 1-32.

[Google Scholar](#)

Hasan, R. U., Prabhat, P., Shafaat, K. and Khan, R. (2013). Phytochemical investigation and evaluation of antioxidant activity of fruit of *Solanum indicum* Linn. *Int. J. Pharm. Pharm. Sci.*, **5** (3): 237-242.

[Google Scholar](#)

Nayar, M. P. and Sastry, A. R. K. (1987). Red data book of Indian plants. Edn. 1st., Botanical Survey of India, Calcutta, pp. 147-159.

[Google Scholar](#)

Nuhu, H.(1999). Pharmacognostic evaluation and toxicity studies of three *Crotalaria species* (Leguminosae). Ph. D. Thesis, Ahmadu Bello University, Zaria, Nigeria.

[Google Scholar](#)

Nuhu, H., Shok, M., Abdurahman, E. M. and Ibrahim, N.D.G.(2000). Alkaloidal composition and toxicity studies of three Nigerian *Crotalaria* Species. *Niger J. Nat. Prod. Med.*, **4**: 43-45.

[Google Scholar](#)

Odey, M. O., Iwara, I. A., Udiba, U. U., Johnson, J. T., Inekwe, U. V., Asenye, M. E. and Victor, O. 2012. Preparation of plant extracts from indigenous medicinal plants. *Int. J. Sci. Technol.*, **1** (12): 688-692.

[Google Scholar](#)

Panda, S. K., Debajyoti, D. and Triphathy, N. K.(2015). Phytochemical investigation and anthelmintic activity of various leaf extracts of *Crotalaria pallida* Aiton. *World J. Pharm. Sci.*,**4**(2): 336-342.

[Google Scholar](#)

Raaman, N.(2006). Phytochemical Techniques. Edn. 1st., New India Publishing Agency, New Delhi, pp.19-24.

[Google Scholar](#)

Ramachandran, V. S. and Nair, V. J.(1988). Flora of Cannanore. Botanical Survey of India, Calcutta, pp. 599.

[Google Scholar](#)

Ramamurthy, V. and Sathiyadevi, M. (2017). Preliminary phytochemical screening of methanol extract of *Indigofera trita* Linn. *J. Mol. Histol. Med. Physiol.*, **2**(1): 1-5.

[Google Scholar](#)

Samba, R. T., Sylla, S. N., Neyra, M., Gueye, M., Dreyfus, B. and Ndoye, I.(2002). Biological nitrogen fixation in *Crotalaria* species estimated using the 15N isotope dilution method. *Afr. J. Biotechnol.*,**1**(1): 17-22.

[Google Scholar](#)

Sanjappa, M.(1991). Legumes of India. Edn. 1st., Bishen Singh Mahendra Pal Singh, Dehra Dun, (4) pp. 338.

[Google Scholar](#)

Silva, G. O., Abeyundara, A. T. and Aponso, M. M.(2017). Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *Am. J. Essent. Oil. Nat. Prod.*, **5** (2): 29-32.

[Google Scholar](#)

Tiwari, P., Kumar, B., Kaur, M., Kaur, G. and Kaur, H.(2011). Phytochemical screening and extraction: A review. *Internat. Pharma. Sci.*,**1**(1): 98-106.

[Google Scholar](#)

