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Preliminary phytochemical investigation of pod and seed extract of *Crotalaria incana* L. subsp. *purpurscens*

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ABSTRACT

Objective: To investigate various phytochemical constituents present in pod and seed of *Crotalaria incana* L. (*C. incana*).

Methods: A number of phytochemical tests were applied to identify the class of compounds in the CH₂Cl₂/CH₃OH (1:1) pod and seed extract of *C. incana*.

Results: Phytochemical screening of the CH₂Cl₂/CH₃OH (1:1) pod and seed extract of *C. incana* subsp. *purpurscens* revealed that the presence of alkaloids, flavonoids, terpenoids, tannins and steroids.

Conclusions: The present work conducted on the CH₂Cl₂:CH₃OH (1:1) pod and seed extract of *C. incana* identified various classes of compounds present on the pod and seed extract.

1. Introduction

The genus *Crotalaria* belongs to the Fabaceae family and contains approximately 600 species that grow wild in tropical and subtropical areas[1]. In Ethiopia, 85 species of *Crotalaria* have been recorded. Whilst about 15 of these species are endemic to the country, the remaining are known to occur in other tropical countries, mainly in Africa. Only few members of the genus have been studied, and the degree and pattern of toxicity was found to vary[2].

Crotalaria incana L. subsp. *purpurscens* (*C. incana*) which is included in Fabaceae family has been used for various traditional medicinal practices such as astringent, jaundice and palpitation, inflammation, skin disease and purgative[3]. In the northern part of Ethiopia, where present study were carried out, the root of *C. incana* was crushed, mixed with water and drunk as traditional medicine for rabies[4], and the seed was taken to treat of wound traditionally by local people[5]. The vernacular name of *C. incana*

is known as “yeayte misir” in Amharic.

In continuation of the ongoing project to study the chemical constituents and biological activity of medicinal plants of Ethiopian flora, we hereby present the phytochemical screening tests of the pod and seed extract of *C. incana* subsp. *purpurscens*.

2. Materials and methods

2.1. Plant material

The pod and seed of *C. incana* were collected in December, 2014 from Amahara Region, South Gondar Zone from Debre Tabor University garden, located around Debre Tabor City, located 666 km from Addis Ababa, the capital of Ethiopia. The plant material was identified by the National Herbarium, Department of Biology, Addis Ababa University, where voucher specimens were deposited.

2.2. Extraction

The plant was dried for two months under shade and then powdered with a mechanical grinder. The dried powder pod and seed of *C. incana* (250 g and 180 g, respectively) were extracted by cold percolation with CH₂Cl₂/CH₃OH (1:1) two times for 24 h while shaking at speed of 220 r/min and temperature controlled at 25.4 °C separately. The extract was concentrated using a rotary evaporator.

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2.3. Preliminary phytochemical screening

Phytochemical screening tests were done to determine the class of compounds present in the crude extract following the standard protocols[6,7]. The results were reported as (+) for presence and (-) for absence in Table 2.

2.3.1. Test for alkaloids

A small portion of the sample was stirred separately with few drops of dilute hydrochloric acid and was tested with Dragendorff's reagent for the presence of alkaloids, and a white to buff precipitate was observed which proved the presence of alkaloids[6,7].

2.3.2. Tests for steroids and terpenoids (Salkowski test)

About 0.2 g of the extract was mixed with 2 mL of chloroform and 3 mL of concentrated sulphuric acid. Red color at lower layer indicated presence of steroids and formation of yellow colored lower layer indicated presence of triterpenoids[6,7].

2.3.3. Test for tannins

A small quantity of the sample was taken separately in water and test for the presence of tannins was carried out with the dilute ferric chloride solution (5%) resulted in a characteristic violet color[6,7].

2.3.4. Test for flavonoids (alkaline reagent test)

About 5 mL of dilute aqueous ammonia solution was added to a 0.2 g of the aqueous filtrate of the plant extract, followed by addition of concentrated H₂SO₄. The instant disappearance of yellow coloration indicated the presence of flavonoids in the crude extract[6,7].

2.3.5. Test for free anthraquinones

About 0.5 g of the extract was boiled with 10% HCl for few minutes in water bath and filtered. The filtrate was allowed to cool and equal volume of CHCl₃ was added to the filtrate. Few drops of 10% ammonia was added to the mixture and heated. The formation of rose-pink colour was taken as an indication for the presence of anthraquinones[7].

3. Results

The percentage yields of pod and seed extracts of *C. incana* were 9.3% and 7.4% (w/w) with brown and yellowish color, respectively. The pod and seed of *C. incana* were used to investigate primarily phytochemical studies. The selected parts of plant were analyzed for phytochemical screening for the extracts obtained from cold extraction successfully using CH₂Cl₂/CH₃OH (1:1). The extracts were subjected to various qualitative tests for phyto-constituents such as alkaloids, flavonoids, triterpenoids, tannins, steroids and anthraquinones. The extracts of pod and seed of *C. incana* reacted positively with Dragendorff's reagent test[6,7], which showed a white precipitate indicating the presence of alkaloids. The extracts have shown positive response to Salkowski test[6,7]. The formation of yellow colored lower layer indicated presence of triterpenoids, and the extracts have shown positive response to alkaline reagent test[6,7]. The disappearing of the yellow color indicated the presence of flavonoids and the extracts have also shown positive response to the dilute ferric chloride

solution[6,7]. Formation of violet color indicated the presence of tannins, and pale red color at lower layer was observed, which also indicated presence of steroids and absence of anthraquinones.

4. Discussion

The plant *C. incana* belonging to the family Fabaceae was taken up in our study to screen and give a report on the possible preliminary phytochemical screening and exhaustive extraction of the plant material was done with CH₂Cl₂/CH₃OH (1:1) and the extracts were screened for the presence of various medicinally active phytoconstituents. The pod and seed extracts of the plant of *C. incana* were subjected to phytochemical screening which reveals the presence of various pharmacological active components. The traditional use of the plant may be attributed to its high contents of alkaloid, tannins, flavonoids, steroids and terpenoids constituents.

This work has identified the phytochemical constituents in pod and seed extracts of *C. incana*. Based on this information, it could be concluded that this plant is natural sources of various bioactive secondary metabolites. The present investigation helps the discovery of plant based drugs to human welfare. Further studies of this plant species should be carried out to investigate the isolation and characterization bioactive compounds in support of its traditional use.

Conflict of interest statement

We declare that we have no conflict of interest.

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