

Phytochemical studies on Ethanolic Extract (Stem, Leaf and Fruit) of Indian Traditional Medicinal Climber "*Cocculus Hirsutus*"

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Cocculus hirsutus is Indian traditional medicinal climber. It is perennial shrub and is commonly found in in and around Mej river catchment area. It is belonging to the family *menispermaceae*. Its various parts are putative to have medicinal properties in folk system of medicine. Qualitative analysis of primary phytochemical from the ethanolic extract of different parts of this plant has been done in this work. The study revealed that they contain sufficient amount of Carbohydrates, Proteins and Lipids.

Keywords: Metabolites, Carbohydrates, Proteins and Lipids.

Introduction

Metabolic substances are an important part of plant life. The metabolites can be mainly divided into two types such as primary metabolites and secondary metabolites. The primary metabolites are essential for the survival of the plants life. Their product is result of the primary metabolic pathways, which include sugars, proteins, amino acids, fatty acids, fats, pyrimidines and purines. Their cells are produced in large amounts. Plants products are most wonderful gift from nature has been used as drugs. Some plant species which are across different ethnic groups various types of drugs are obtained from are known as medicinal plants (Yadav *et al*, 2010).

Cocculus hirsutus (Family *Menispermaceae*) is climbing or prostrate, much branched, perennial herb commonly known as *Broom creeper* or *Ink berry* in English and *Patagarudi* in Hindi. It also possess many medicinal properties. *Cocculus hirsutus* is a weak scandent, dioecious shrubs climber contains alternate leaves with (1.5-8.0 x 0.7-4.5 cm,) ovate-oblong, cordate-lanceolate or subdeltoid. Flowers are minute, yellowish green in colour. Male flowers in small axillary, cymose panicles and Female flowers in axillary clusters, rarely racemose. Fruits are Drupes smooth, reddish purple type. Their flowering and fruiting time is September to April. It is distributed on the fringes of forests and in wastelands on the plains of India.

The present research paper deals and qualitative test of the ethanolic extracts of climber *Cocculus hirsutus* for primary metabolites.

Aim of the Study

1. To prepare extract of different parts (leaves, stem, fruit) of *Cocculus hirsutus* on organic solvent ethanol.
2. Identification of primary metabolite in the extract to facilitate further study for human welfare.

Material and Methods

Plant collection

Cocculus hirsutus was collected from in and around catchment area of Mej River. The identity of the plant species was established by Herbarium chamber Government College, Bundi by author department of botany. The herbarium sheets were prepared according to the standard method suggested by Jain and Rao (1977).

Preparation of plant extract

Fresh leaves stem and fruit of *Cocculus hirsutus* were washed thoroughly tap water and were dried in hot air oven at 40-50° c for a week. 60gm of dried powder was extracted for 24 hours in 300 ml solvent (ethanol 99%). Repeated extraction was done with the some solvent till



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colourless solvent was obtained. The condensed extract was used for screening of primary metabolites. Soxhlet equipment was used in this study. Powdered plant material (60 g) was extracted with organic solvents (300 ml) such as n-hexane, ethyl acetate methanol and ethanol in Soxhlet apparatus (Raaman, 2006)

Primary Metabolites Analysis**Test for Carbohydrates****Molisch test**

The test was carried out by following the method Ramakrishanan *et al.* 1994. 2 ml of aliquot of the extract was treated with 2 drops of Molisch reagent. After shaking and holding test tube in slanting position 2 ml concentrate Sulphuric acid along the side of the test tube. The reddish violet ring at the junction of two solutions indicates presence of Carbohydrates.

Benedict's test

1 ml of aliquot of the extract was treated with 3 ml of Benedict's reagent in a test tube and boiled for 10 minutes. Bluish or yellowish orange precipitate indicates the presence of reducing sugar.

Test for Proteins**Millon's test**

The test was carried out by following the method Fisher, 1968; Ruthmann, 1970. 2 ml of aliquot of the extract was treated with 2 drops of Millon's reagent in a test tube. The test tube a white creamy precipitate appeared which changed to brick red on heating. It indicates the presence of Proteins.

Biuret test

The test was carried out by following the method Gahan, 1984. An aliquot of 2 ml of filtrate is treated with few drops of copper sulphate solution. To this, 1 ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink colour in the ethanolic layer indicates the presence of Proteins.

Test for Fats and fixed oils**Saponification test**

The test was carried out by following the method Kokate, (1999). A few drops of 0.5 N alcoholic potassium hydroxide solutions are added to a small quantity of extract along with a drop of phenolphthalein. The mixture is heated on water bath for 2 hours. Formation of Soap or partial neutralization of alkali indicates the presence of fixed oil.

PLATE 20 *Cocculus hirsutus* (L.) W. Theob.



Family- Manispermaceae B.N.- *Cocculus hirsutus* (L.) W. Theob. V.N.- *Chhireta*



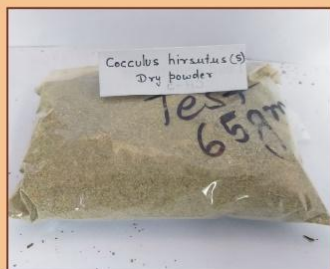
DRY LEAVES



DRY STEMS



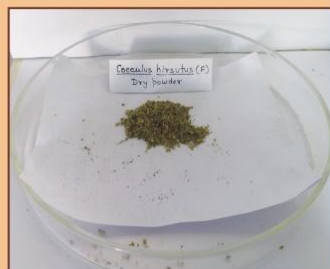
FRUITS



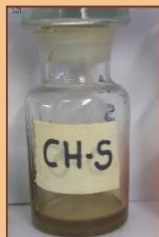
LEAF POWDER



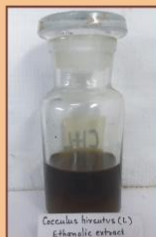
STEM POWDER



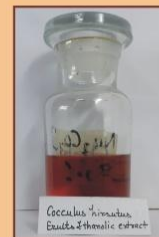
FRUIT POWDER



LEAF EXTRACT



STEM EXTRACT



FRUIT EXTRACT

Result

Results of qualitative analysis of primary metabolites (carbohydrates, proteins and fats and fixed oils) have been presented in Table 1 and Plate. The results of the preliminary analysis of *Cocculus hirsutus* show that the primary metabolites are present in fruits, leaves and stems. The Molisch's test to ascertain presence of carbohydrates for leaf and fruit extracts revealed higher degree of precipitation (+++), whereas the same test for stem extract resulted in moderate degree of precipitation (++) . Benedict's test for reducing sugars showed moderate degree of precipitation (++) in stem and fruit

extracts followed by lesser degree of precipitation (+) in leaf extract. Leaf and fruit extracts showed higher degree of precipitation (+++), when subjected to Millon's test. The ethanolic extract of stem however, showed lesser degree of precipitation (+) in the same test. Biuret test resulted in higher degree of precipitation (+++) in all three plant extracts, namely, leaf, stem and fruit. Saponification test showed the presence of fats and fixed oils with moderate degree of precipitation (++) in fruit and leaf of extracts whereas lesser degree of precipitation (+) in stem extract has been recorded for the same plant species.

Table 1 Qualitative analysis for primary metabolites in alcoholic leaf, stem and fruit extracts of *Cocculus hirsutus*

S.No.	Name of Phytochemicals	Name of tests	Extracted Part	Results
1	Carbohydrates	Molisch's Test	Leaf	+++
			Stem	++
			Fruit	+++
2.	Reducing sugars	Benedict's Test	Leaf	+
			Stem	++
			Fruit	++
3	Proteins	Millon's Test	Leaf	+++
			Stem	+
			Fruit	+++
		Biuret Test	Leaf	+++
			Stem	+++
			Fruit	+++
4	Fats and Fixed Oils	Saponification Test	Leaf	++
			Stem	+
			Fruit	++

PLATE 28
Phytochemical screening of primary metabolites of *Cocculus hirsutus*

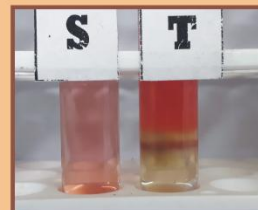
Detection of Carbohydrates



Molisch's Test - Leaf



Molisch's Test - Stem



Molisch's Test - Fruit

Detection of Reducing Sugars



Benedict's Test - Leaf



Benedict's Test - Stem



Benedict's Test - Fruit

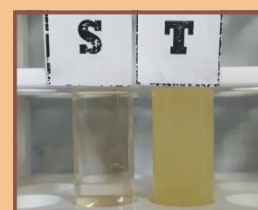
Detection of Proteins



Million's Test - Leaf



Million's Test - Stem



Million's Test - Fruit



Biuret Test - Leaf



Biuret Test - Stem



Biuret Test - Fruit

Detection of Fats and Fixed Oils



Saponification Test - Leaf



Saponification Test - Stem



Saponification Test - Fruit

Discussion

The presence of carbohydrates was ascertained by Molisch test. The result reveals that fruits and leaves extract has more quantity of carbohydrates as it exhibited higher degree of precipitation (+++). However the stem extracts showed moderate degree (++) of precipitation.

The presence of reducing sugars was ascertained by Benedict test. The result reveals that fruits and stem extract has more quantity of carbohydrates as it exhibited moderate degree of precipitation (++) . However the leaves extracts showed lesser degree (+) of precipitation.

The presence of protein in the extract was ascertained by Millon test and Biuret test. Fruits, Stem and Leaves extract exhibited presence of protein with higher degree of precipitation (+++) in Biuret test. Fruits, and Leaves extract exhibited presence of protein with higher degree of precipitation (+++) in Millon test, However stem extract showed presence of protein with lesser degree of precipitation (+) in Millon test.

Saponification test indicates the presence of Fats and fixed oils with moderate degree of precipitation (++) in fruit extract and leaves whereas the same test resulted in lesser degree of precipitation (+) for the stem extract.

Present findings are supported by work of a number of researchers who also carried out phytochemical analysis studies of *Cocculus hirsutus*. Jangir *et al.* (2016) in a review provided updated comprehensive information of pharmacological properties and phytochemistry of *Cocculus pendulus*. This medicinal plant is used to cure leprosy, helminthic, syphilis, fever, menstrual disorders, etc. Patil *et al.* (2014) in a phytochemical and aphrodisiac study of *Cocculus hirsutus* areal parts (stem and leaf) extracts observed the extract to have promotory effects on spermatogenesis and performance of accessory reproductive organs in albino rats. Kalirajan *et al.* (2012) tested wound healing and antimicrobial properties of methanol and aqueous extract of *Cocculus hirsutus*. Savithramma *et al.* (2011) screened fresh leaves of 20 different medicinal plants for secondary metabolites by qualitative phytochemical analysis and reported presence of various secondary metabolites including triterpenoids, steroids, saponins, tannins, phenols, flavonoids, etc. Nayak and Singhai (2003) examined antimicrobial properties of roots of *Cocculus hirsutus* using ethanolic and petroleum ether extracts. Kumar *et al.* (2012) were presented a detailed pharmacognostic study of the leaf of *Cayratia trifolia*. Present finding are supported similar research worked by Prasad & Sharma (2020), Prasad & Sharma (2019), Prasad & Sharma (2018), Bhaduria *et al.*, (2012), Rahman *et al.*, (2015) and Deokate & Khadabadi, (2012). They evaluated different climber species which also support present research work.

Conclusion

Present research highlights the presence of primary metabolite like carbohydrates, protein and lipid in *Cocculus hirsutus*. Beneficial properties could

be done in further study by qualitative assessment of this climber.

Acknowledgement

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