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ANTI OXIDANT ACTIVITY OF THE ROOT OF ETHANOLIC EXTRACTS OF *SWERTIA CHIRATA* LINN.

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ABSTRACT

Swertia Chirata is a belongs family Gentianaceae used in folk medicine for antipyretic, anthelminitic, antiperiodic, cathartic and in asthma and leucorrhoea in Ayurveda and as harsh, analeptic, stomachic, mitigate inflammation, relaxing to pregnant uterus and never ending fever. The purpose of the present study is to investigate the anti oxidant activity of the ethanolic extract of roots of plant swertia chirata .the antioxidant activity of combined ethanolic extract was done by using the DPPH method against the standard ascorbic acid. Here the extract shows activity at higher concentration against the standard ascorbic acid.

KEYWORDS: Swertia Chirata anti oxidant activity, DPPH activity.

INTRODUCTION

Herbs orchestrate resurgence and vegetal awakening is supervened everywhere in the world. Vegetal commodities currently illustrate assurance as compared to the factitious ones that are contemplate as alarming to humans and environment. Out of 2,50,000 higher plant species on this planet, more than 80,000 types are declared to have in some ways remedial importance and around 5000 species have characteristic analeptic value. Organized storage and commodious plowing of relevant medicinal plant species are thus of ample precedence. An important herb Swertia chirayita, is a medicinal plant aboriginal to clement Himalayas in India, Nepal and Bhutan. Its medicinal usage is declared in American and British pharmacopoeias, Indian Pharmaceutical codex and in different conventional systems of medicines like Ayurvedic, Unani and Sidha. Plants mainly utitize in Ayurveda can contribute organically active compounds and lead structures for the advancement of transformed subordinates with increased activeness and abate virulence. We are well enumerate as the most paramount chirayita producer and vendor based in India. The chief bioactives of Swertia are Xanthones, other active constituents of this genus are the secondary metabolites which played a momentous role in biological activities like being hepatoprotective, digestive, astringent, laxative, anti-inflammatory and anti-malarial. Hence this herb provides potent therapeutic lead compounds, which would be beneficial for mankind.

MATERIALS AND METHODS

Drying and size reduction: Bark part of the two plants were carefully shade dried for 15 days. To ensure complete dryness they were kept in hot air oven at 45˚C for 5 minutes. Then they are subjected to size reduction to make powder by using mechanical grinder. The crushed mass of bark was then carried out for the process of extraction.

Extraction procedure[12]

1. 800gms of the air-dried powdered plant material extracted with ethanol in soxhlet extractor.
2. Soxhalation of leaf powder with ethanol for 24hrs to obtain the product.
3. Then the dried marc is extracted with water by decoction.
4. Concentrate the extract by distilling of the solvent and then evaporating to dryness on the water-bath.

Preliminary Phytochemical Analysis[13,14,15,16]:

Test for Tannins

1. A small portion of extract was treated with 5%ferric chloride solution. Appearance of green to blue color was taken as a positive test for tannins.
2. Small portion of extract was treated with lead acetate. Appearance of creamy precipitate was considered as a positive test for tannins.

Test for Alkaloids

1. **Mayer's Test:** The Extract to be tested is treated with few drops of dilute 2N HCL and 0.5 ml

Mayer's reagent .White precipitate was obtained which confirm the presence of alkaloids.

2. **Wagner's Test:** The extract is treated with few drops of 2N HCL and 0.5 ml Wagner's reagent. Brown flocculent precipitate was obtained which confirm the presence of alkaloids.
3. **Hager's Test**: The extract is treated with few drops of dilute 2N HCL and 0.5 ml Hager's reagent. Yellow colored precipitate was obtained which confirms the presence of alkaloids.

Test for Steroids

1. **Salkowski reaction:** To 2ml.of extract, add 2ml of chloroform and 2ml.conc.H_2SO_4. Shake well, Chloroform layer appears red and acid layer shows greenish yellow fluorescence.
2. **Liebermann-Burchard reaction:** Mix 2 ml extract with chloroform. Add 1-2ml.aceteic anhydride and 2 drops of conc.H_2SO_4 from the side of test tube.
3. **Libermann's reaction:** mix 3ml.extract with 3ml. acetic anhydride. Heat and cool. Add few drops conc. H_2SO_4. Blue color appears.

Tests for Glycosides

1. **Borntrager's test:** About 50mg of extract was hydrolysed with 2ml of concentrated HCl for 2hrs on water bath and filtered. To 2ml of filtrate hydrolysate, 3ml of $CHCl_3$ was added and shaken. $CHCl_3$ layer was separated and 10% NH_3 solution was added. Formation of pink colour indicates the presence of anthraquinone glycosides.
2. **Baljet's test:** The alcoholic or aqueous extract test solution is treated with sodium picrate. Appearance of yellow to orange colour indicates the presence of glycosides.
3. **Keller-Kiliani test:** About 2ml of test solution is treated with few drops of ferric chloride solution and mixed and then sulphuric acid containing ferric chloride solution is added, it forms two layers. Appearance of lower layer in reddish brown and upper layer in bluish green indicates the presence of glycosides**.**

Test for Saponins

Foam's test: A small amount of dry extract was boiled with water and allowed to cool. It was then shaken vigorously for a minute. The formation of persistent honey comb like froth was considered as a positive test for saponins.

Test for Sugars

1. **Molisch's test:** It was performed for the presence of carbohydrates. 1 ml of 10%alcoholic solution of α-napthol was added to the extract and mixed. Then 1ml of concentrated sulphuric acid was carefully poured along the sides of the test tube violet ring formed at the junction which is considered positive test for carbohydrates.
2. **Fehling's test:** 5ml of solution of extract was heated with equal volumes of Fehling's solution A & B. Transition of color from blue through green to reddish orange confirms the presence of reducing sugars.
3. **Benedict's test:** 5 ml of solution of the extract was heated with 5 ml of Benedict's reagent .A green, yellow or orange red precipitate was considered as a positive test for reducing sugars.

Test for Proteins

1. **Biuret test:** A small portion of extract was treated with Biuret reagent.
2. **Xanthoprotein test:** Mix 3ml. T.S. with 1ml.conc. H_2SO_4. White precipitate is formed. Boil. Solution turns black or brownish due to Lead sulphide formation.

Pharmacological evolution

Antioxidant activity by DPPH method[20,21]

Antioxidant behaviour of the extracted compound was measured *in vitro* by the inhibition of generated stable 2,2-diphenyl- 1-picrylhydrazyl (DPPH) free radical. Methods vary greatly as to the generated radical, the reproducibility of the generation process, and the end point that is used for the determination. The DPPH solution was prepared by dissolving accurately weighed 22 mg of DPPH in 100 ml of ethanol. From this stock solution, 18 ml was diluted to 100 ml with ethanol to obtain 100 µM DPPH solutions. The sample solution was prepared by accurately weighed 2.1 mg of each of the compounds and dissolved in 1 ml of freshly distilled DMSO separately to obtain solutions of 2.1 mg/ml concentration and the standard solution of was prepared by accurately weighed 10.5 mg of α-Tocopherol and dissolved in 1 ml of freshly distilled DMSO to get 10.5 mg/ml concentration.

A different concentration of extract was prepared by the addition of ethanolic solution of DPPH radical. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm against the corresponding blank solution. The final concentration of the samples and standard α-Tocopherol solutions used is 100µg/ml. The percentage scavenging DPPH radical inhibitions were calculated by using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Where, Abs control was the absorbance of DPPH radical and ethanol, Abs sample was the absorbance of DPPH radical and sample/standard.

The scavenging activity was expressed in terms of IC50, the concentration of the samples required to give a 50% reduction in the intensity of the signal of the DPPH radical. The results were done at least in triplicate.

RESULTS

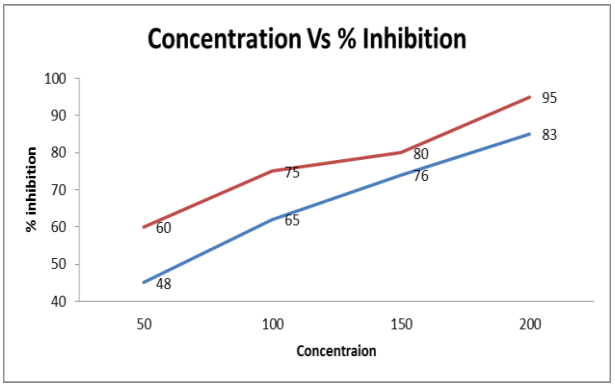
Table 1: Phytochemical constituents present in total ethanolic extract of Swertia Chirata.

Tests	Swertia Chirata
1. Tannins	+
2. Alkaloids	-
3. Steroids	+
4. Glycosides Cardiac Anthraquinone Saponin Flavanoids Coumarins	 + - - + -
5. Sugars	+
6. Protiens	+

(+) - Indicates the presence of the phyto constituent
(-) - Indicates the absence of the phyto constituent

Anti oxidant activity

S. No	Compound	Concentration	% inhibition
1	Ethanolic extract	50	48
2		100	65
3		150	76
4		200	83
5	Standard (Ascorbic acid)	50	60
6		100	75
7		150	80
8		200	95



DISCUSSION

The ethanolic extract of the two plants were Phyto chemical evolution was done and anti oxidant activity made by using the DPPH reagent method .here the concentration in µg/ ml at 50,100,150,200 shows the % inhibition at 50, 62,74,85 respectively. These results were compared with the standard ascorbic acid.

CONCLUSION

The present study has revealed that the extracts that have been prepared from the leaves of *Swertia Chirata* have shown the significant Anti oxidant activity. The antioxidant activity of Ethanolic extract of leaves of *Swertia Chirata* shows less activity than that of standard drug.

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