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QUALITATIVE PHYTOCHEMICAL SCREENING, TOTAL PHENOLIC CONTENT, *IN-VITRO* ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY IN METHANOLIC EXTRACTS OF *CANTHARELLUS CIBARIUS* FR.

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Abstract

Cantharellus cibarius, the golden chanterelle is one of the most well-known wild edible mushrooms belongs to family Cantharellaceae. Qualitative phytochemical analysis of *C. cibarius* extracts showed the presence of alkaloids, anthraquinone, glycosides, tannins, saponins, flavonoids, terpenoids, phenolics, carbohydrates as well as proteins and amino acids. Anthocyanins and sterols were not found. The total phenolic content was 194.31 (mg Gallic acid equivalents per gram weight). The results obtained from both the methods revealed that *Cantharellus cibarius* exhibits high antioxidant activity. At the concentration of 500µg/ml, the extracts showed 92.2% and 91.1% scavenging activity by DPPH and hydrogen peroxide method, respectively. The methanolic extracts of *L. sanguifluus* were found to have significant antimicrobial activity against all the three test microorganisms *i.e. Escherchia coli*, *Pseudomonas aeroginosa* and *Candida albicans*. The findings suggest that *C. cibarius* methanolic extracts have very good antioxidant and antimicrobial activity and can be used as complete functional food.

Key words : *Cantharellus cibarius,* phytochemical screening, DPPH, hydrogen peroxide, anti-microbial.

Introduction

Mushrooms are the fungi that have been used as food since times immemorial (Rahi *et al*., 2004). Wild edible mushrooms fall under the category of non-timber forest products (NTFP), which have been untapped resources because a wide variety of wild mushrooms are still unexploited (Devkota, 2008). These are one of the treasures of nature with a prolonged history of use as food and also for their medicinal benefits. Mushrooms have long history of medicinal usages in addition to their nutritional value (Crisan and Sands, 1978). The consumption of mushrooms is more for their flavor than for nutritive elements; nevertheless, the mushrooms possess good amount of proteins and vitamins and this has been the reason for great mushroom growing industry development during the past few decades and it is constantly growing proving thereby that people now appreciate mushrooms as food.

Cantharellus cibarius also commonly known as the chanterelle, golden chanterelle, girolle, pfifferling or gallinacci is the best known specie of the genus *Cantharellus.* The name *Cantharellus* is derived from the Latin word 'cantharus' meaning a large drinking vessel, a bowl, or chalice. The sobriquet "cibarius" comes from the Latin 'cibus' meaning food (or rations). It is one of the most well-known wild edible mushrooms and belongs to family Cantharellaceae. Chanterelles are mycorrhizal with hardwoods, meaning they associate with trees and possibly some other bushes or plants and can occasionally occur in groups in mixed woodlands. It is fairly common and easy to spot as they grow in great profusion every year in the same place (plate A).

As a culinary-medicinal mushroom, *C. cibarius* has been used as a good source of food due to its rich flavor, as potential source of nutraceuticals and in dietary supplements. This mushroom can be cooked in many ways. Young fruiting bodies are picked are giving real delicacy. It is an important mushroom because it grows in great quantities and is not usually eaten by insects (Dermek, 1982). From India, many species of edible fungi has been reported to be traditionally and regularly consumed by the local inhabitants without causality and fatality. Wild edible mushrooms having very good commercial value are species of *Morchella*, *Helvella*, *Hericium*, *Sparassis*, *Hydnum*, *Trapeinda*, *Clavaria*, *Ramaria*, *Boletus*, *Albatrellus*, *Cordyceps*, *Lactarius* and *Rusulla* species (Lakhanpal, 2000). All these wild

edible mushrooms are consumed as well as sold fresh, collected and dried for sale (Lakhanpal and Rana, 2005; Lakhanpal *et al*., 2010).

It is prized for cooking throughout Europe and North America, although it is far more popular in Europe (Fischer and Bessette, 1992). The fruiting bodies possess mild fruity aroma that smells of apricot as it dries. It has excellent flavour is slightly peppery and needs long slow cooking, as it can be tough. The texture is reported to improve, if fructifications are shredded and soaked in milk overnight, before cooking. It is rich in vitamins C (Barros *et al.*, 2008), A and D, and in the pigment carotene. *C. cibarius* is popularly consumed for its nutritive value and its unique flavour (Pilz *et al*., 2003). It has a nutritional profile that can profoundly improve health. The medicinal benefits of *Cantharellus cibarius* are derived from its component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body. The extracts of the fungi (both methanolic and ethanolic) were found to contain phenols, terpenoids, flavonoids, alkaloids, anthraquinones and saponin (Aina *et al*., 2012). A study by Egwim *et al*. (2011) also revealed the presence of varying quantities of alkaloids, flavonoids, saponins, tannins with absence of anthraquinones and steroids. A phytochemical screening conducted a few years later by Hamzah *et al.* (2014) revealed the presence of steroids, alkaloids, cardiac glycosides, saponins, flavonoids, terpenes, tannins (including phlobatannins) and phenolics in the *Cantharellus cibarius* extracts in varying proportions. The present study evaluates the quantitative phytochemicals, total phenolic content, *in-vitro* antioxidant and antimicrobial activity of *C. cibarius* methanolic extracts so that it can be used as complete Functional food.

Materials and Methods

Chemicals

Ethanol, Methanol, Sodium carbonate, Gallic acid, Folin-Ciocalteu reagent and DPPH (1,1-diphenyl-2-picrylhydrazyl) were used. All the chemicals and reagents used were of analytical grade.

Microbial cultures

Microorganism slant cultures were obtained from MTCC, IMTECH Chandigarh - 160 036, India. The following cultures were used for the study:

- *Escherchia coli* (MTCC1698),
- *Pseudomonas aeroginosa* (MTCC6458), and
- *Candida albicans* (MTCC7315).

Plate A : Fruiting body of *C. cibarius*.

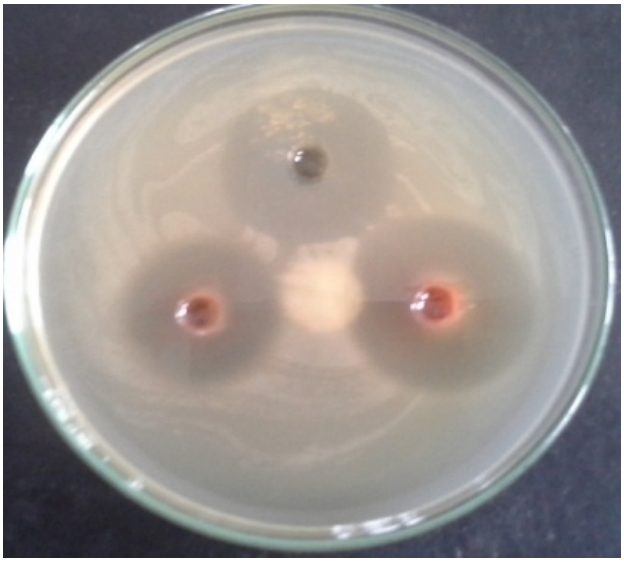

Plate B : Antimicrobial activity of *Cantharellus cibarius* against *Escherchia coli.*

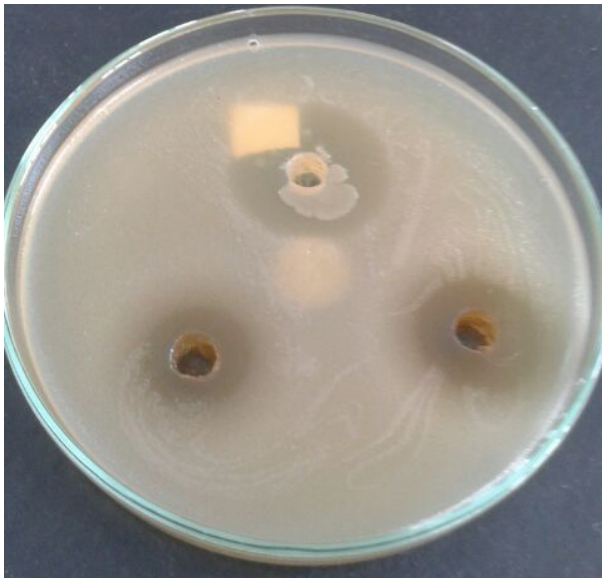

Plate C : Antimicrobial activity of *Cantharellus cibarius* against *Pseudomonas aeruginosa.*

Plate D : Antimicrobial activity of *Cantharellus cibarius* against *Candida albicans*.

Collection of plant material and sample preparation

The wild edible mushroom specie was collected from the Northwest Himalayan region of Shimla, India (31°6'12"N77°10'20"E). The fruiting bodies were thoroughly cleaned of extraneous matter and dried completely and coarsely grounded.

Preparation of extracts

Grounded mushroom was extracted with methanol at room temperature prior to removal of solvent. 10 grams of the ground sample was mixed with six times of 99.6% methanol and kept for 24 hour. This process was repeated thrice and filtrates were collected. The filtrates obtained were concentrated under vacuum on a rotary evaporator (Buchi Rotary Evaporator, Model R-124) and stored at 4°C for further use (Song *et al*., 2010).

Quantitative phytochemical screening

Methanolic extracts of *C. cibarius* were used for qualitative screening of phytochemicals as per standard biochemical procedures. The preliminary tests for methanol extracts were performed to confirm the presence of alkaloids, anthraquinones, anthocyanins, carbohydrates, flavonoids, glycosides, phenols, proteins and amino acids, saponins, steroids, tannins and terpenoids (Tiwari *et al*., 2011).

Estimation of total phenolic content

The total phenolic content in methanolic extracts of grounded sample was estimated by Folin-Ciocalteu reagent, as described by Singleton and Rossi (1965). 100 mg of gallic acid was dissolved in 100 ml ethanol to prepare gallic acid stock solution (1000 µg/ml). Various dilutions of standard gallic acid were prepared from this stock solution. 1 ml aliquots of 1.0, 2.5, 5.0, 10, 25, 50 and 100 µg/ml of gallic acid solution were mixed with 5.0 ml of Folin-Ciocalteu reagent (diluted ten-fold) and 4.0 ml of sodium carbonate solution (75 g/l) and calibration curve was plotted. The absorbance was measured after 30 min at 20°C at 765 nm. 1 ml extract was mixed separately with the same reagents and absorbance was measured at 765 nm after 1 hour. The total phenolic compound in the extract was determined using the formula:

$$C = C_1 \times V/m$$

C = Total content of phenolic compounds in mg/g in GAE (Gallic acid equivalent); C_1 = The concentration of gallic acid established from the standard curve in mg/ml; V = The volume of extract in ml, M = Weight of extract in grams.

***In-vitro* evaluation of antioxidant activity**

In-vitro antioxidant activity of the extract of *C. cibarius* was determined by using two different methods: Free radical scavenging activity using DPPH method and free radical scavenging activity using hydrogen peroxide.

(i) Free radical scavenging activity using DPPH method

The free radical scavenging activities of sample extract was measured by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Kaur *et al*., 2008). Briefly, 0.1 mM solution of DPPH in ethanol and 1.5 ml of this solution was added to 0.5 ml of extract solution in ethanol at different concentrations (50-300 µl/ml). The mixture was shaken and allowed to stand at room temperature for 30 minutes. The absorbance was then measured at 517nm using a spectrophotometer. A blank without DPPH was used to remove the influence of the color of the extracts and an ethanolic solution of DPPH was used as a negative control. Ascorbic acid was used as a reference. All of the measures were carried out in triplicates. The DPPH radical scavenging activity was calculated using the following equation:

$$\% \text{ Scavenging effect} = \frac{A_o - A_s}{A_o} \times 100$$

Where,

A_o = Absorbance of negative control

A_s = Absorbance of sample

(ii) Free radical scavenging activity using hydrogen peroxide

The free radical scavenging activity of sample extract was determined by using hydrogen peroxide (Mohamad *et al*., 2010). An aliquot of 0.6 ml of hydrogen peroxide and 1.0 ml of various concentrations of the extract prepared using phosphate buffer (200-400 micro gm/ml) were mixed followed by 2.4 ml of 0.1 M phosphate buffer (pH 7.4). The resulting solution was kept for 10 minutes

and the absorbance was recorded at 230 nm. All measures were repeated triplicate. For each concentration, mixture without sample was taken as a control and a mixture without hydrogen peroxide was taken as a blank. Ascorbic acid was used as a standard compound. The percentage scavenging activity of hydrogen peroxide was calculated as:

$$\text{Scavenging activity (\%)} = \frac{A_o - A_s}{A_s} \times 100$$

Where,

A_o = Absorbance of negative control

A_s = Absorbance of sample

***In-vitro* anti-microbial activity**

The *in vitro* antimicrobial activity was carried out by Disc diffusion method. The microbial suspension of different dilutions (10^0, 10^{-1}, 10^{-2} and 10^{-3}) was prepared in 0.85% saline and 100 µl of 10^0 and 10^{-3} dilutions were spread evenly on separate Nutrient Agar (NA) Plates. The extracts both diluted (D) and concentrated (C) were used. The dilution of the extracts was done with DMSO (Di-methyl sulfoxide). Then the blank sterile discs containing 20 µl of the sample extract were placed on these PDA plates. The experiment was carried out in triplicates. The plates were then incubated in an incubator at 37°C for about 24 hours and the plates were observed for zone of inhibition and the diameter of the zone of inhibition was calculated for all the plates. Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition around the extract (Duyilemi and Lawal, 2009). The assay was repeated three replicates and the results were recorded as mean ± SD.

Results and Discussion

Qualitative phytochemical analysis of *Cantharellus cibarius* extracts showed the presence of alkaloids, anthraquonone, glycosides, tannins, saponins, flavonoids, terpenoids, phenolics, carbohydrates as well as proteins and amino acids (table 1). Anthocyanins and sterols were not found. The results revealed that *C. cibarius* may be used as potential sources of phytochemicals and thus can be used for designing drugs that can prove to be of keen interest in the treatment and prevention of diseases like cancer, tumor, heart diseases, etc.

The amount of total phenols was determined with Folin-Ciocalteu reagent. Gallic acid was used as standard compound. The standard curve of Gallic acid concentrations and absorbance is shown in fig. 1. The absorbance for various dilutions of gallic acid with Folin-Ciocalteu reagent and sodium carbonate were found.

Table 1 : Qualitative phytochemical screening of *C. cibarius* methanolic extracts.

S. no.	Phytochemical	*Cantharellus cibarius*
1.	Alkaloids	+++
2.	Anthraquinone	++
3.	Anthocyanins	-
4.	Proteins and amino acids	+++
5.	Carbohydrates	+++
6.	Phenols	++
7.	Terpenoids	+++
8.	Sterols	-
9.	Saponins	+++
10.	Glycosides	++
11.	Flavonoids	++
12.	Tannins	++

Note: (+ + +): Shows that phytochemicals are abundantly present, (+ +): Shows that phytochemicals are moderately present, (-): Shows that phytochemicals are absent.

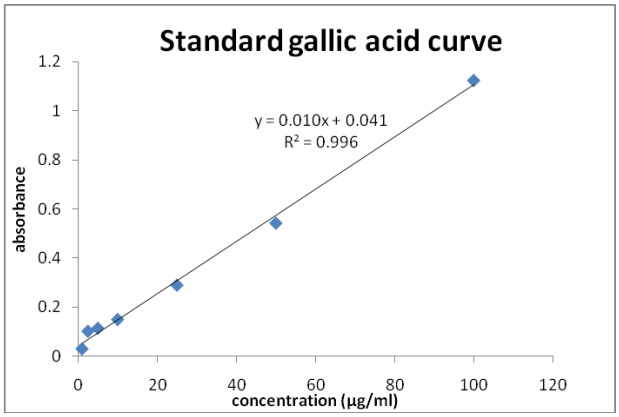

Fig. 1 : Standard gallic acid curve.

Table 2 : Total phenolic content of *C. cibarius* methanolic extracts.

S. no.	Sample	Absorbance at 765nm (mean ± standard error)	Total phenolic content (mg gallic acid equivalents per gram weight)
1.	*C. cibarius*	0.861 ± 0.0012	194.31

Table 3 : Percentage scavenging activity of the *C. cibarius* methanolic extracts at different concentrations at 765 nm using DPPH method.

Concentration (µg/ml)	Absorbance of *C. cibarius*	Per cent scavenging activity
100	0.491	40.19
200	0.347	57.7
300	0.195	76.2
400	0.098	88.0
500	0.064	92.2

Table 4 : Per cent scavenging activity of the *C. cibarius* methanolic extracts at different concentrations using hydrogen peroxide at 230 nm.

Concentration (µg/ml)	Absorbance of *C. cibarius*	Per cent scavenging activity
100	0.480	40
200	0.330	58.8
300	0.183	77.1
400	0.158	80.2
500	0.071	91.1

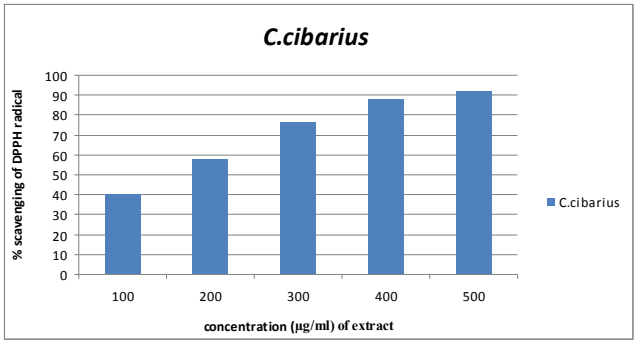

Fig. 2 : Percentage scavenging of DPPH Radical of *C. cibarius*.

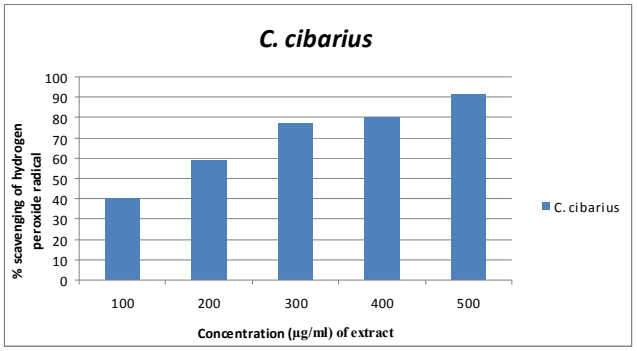

Fig. 3 : Percentage scavenging of hydrogen peroxide radical of *C. cibarius*.

Table 5 : Antimicrobial activity of *C. cibarius* extracts against *E. coli*, *P. aeruginosa* and *C. albicans*.

S. no.	Sample extracts	Zone of inhibition (mm)		
		E. coli	*P. aeruginosa*	*C. albicans*
1.	*C. cibarius*	29 ± 1.328 (C) 28 ± 1.267 (D)	25 ± 1.320 (C) 12 ± 1.552 (D)	32 ± 0.381(C) 30 ± 1.224 (D)

Found standard curve equation was;

$Y = 0.0106 x + 0.041$

$R^2 = 0.996$

The total phenolic content of methanol extracts of *C. cibarius* was 194.31 (mg gallic acid equivalents per gram weight) (table 2). Data expressed as mean ± standard error of three samples analyzed separately.

Antioxidant activity of sample extract at different concentrations was determined using two different techniques *viz*. DPPH method and H_2O_2 method. The DPPH is a stable organic free radical with an absorption maximum band around 515-528 nm (Stankovic, 2011) and is widely used for evaluation of antioxidant potential of compounds. The methanolic extracts of *Cantharellus cibarius* showed good antioxidant activity as evaluated by both the methods. Results are presented in tables 3 and 4.

The results obtained from both the methods revealed that *C. cibarius* exhibits high antioxidant activity. At the concentration of 500µg/ml, the extracts showed 92.2% and 91.1% scavenging activity by DPPH and hydrogen peroxide method respectively. The results are represented by figs. 2 and 3.

The methanolic extract of *Cantharellus cibarius* was found to have significant antimicrobial activity against all the test micro-organisms i.e. *E. coli, P. aeroginosa* and *C. albicans*. The zones of inhibition shown by concentrated (C) and diluted (D) methanolic extracts of the mushroom are presented in Table 5. Highest activity was shown against *C. albicans* followed by *E. coli* and *P. aeruginosa*. Plates B, C and D show the antimicrobial activities of *C. cibarius* against *E. coli, P. aeruginosa* and *C. albicans*, respectively.

References

Aina, D. A., S. G. Jonathan, O. J. Olawuyi, D. O. Ojelabi and B. M. Durowoju (2012). Antioxidant, antimicrobial and phytochemical properties of alcoholic extracts of *Cantharellus cibarius* – a Nigerian mushroom. *N Y Sci. J.*, **5(10)** : 114-120.

Barros, L., B. A. Venturini, P. Baptista, L. M. Estevinho and I. C. F. R. Ferreira (2008). Chemical composition and biological properties of Portuguese wild mushrooms : a comprehensive study. *J. Agri. Food Chem.*, **56 (10)** : 3856–3862.

Crisan, E. V. and A. Sands (1978). Nutritional value. The biology and cultivation of edible mushrooms. Chang, S. T.; Hayes, W. A. Eds.; Academic Press : London. pp. 137-168.

Dermek, A. (1982). *Mushrooms and other fungi*. Galley Press. England. pp. 223.

Devkota, S. (2008). Distribution and status of highland mushrooms : a study from Dolpa, Nepal. *J. Nat. History Mushrooms*, **23** : 51-59.

Duyilemi, O. P. and I. O. Lawal (2009). Antibacterial activity and phytochemical screening of *Chrysophyllum albidum* leaves. *Asian J. Food Agro-Indus.*, S75-S79.

Egwim, E. C., R. C. Elem and R. U. Egwuche (2011). Proximate composition, phytochemical screening and antioxidant

activity of ten selected wild edible Nigerian mushrooms. *American J. Food Nutr*., **1(2)** : 29-94.

Fischer, D. H. and A. Bessette (1992). Edible Wild Mushrooms of North America : a Field-to-Kitchen Guide. Austin, Texas: University of Texas Press.

Hamzah, R. U., A. A. Jigam, H. M. Makun and E. C. Egwim (2014). Phytochemical screening and antioxidant activity of methanolic extract of selected wild edible Nigerian mushrooms. *Asian Pacific J. Trop. Disease*, **4(1)** : S153-S157.

Kaur, P., B. Singh, S. Kumar and S. Kaur (2008). *In vitro* evaluation of free radical scavenging activity of *Rubia cordifolia* L. *J. Chinese Cli. Med*., **3(5)** : 278-284.

Lakhanpal, T. N. (2000). *Wild and cultivated mushrooms*. Natural Resources and Development in Himalaya (ed. L.R. Verma). Malhotra Publishing House, New Delhi, pp 402-417.

Lakhanpal, T. N., O. Shad and M. Rana (2010). *Biology of Indian Morels*. I K International Publishing House, pp. 266.

Mohamad, A., M. Seyed, F. Seyed, B. Fatehmeh and R. Ahmad (2010). Antioxidant and free radical scavenging activity of *H. officinais* H, var. *Angustifolia*, *V. odorata*; *B. hyrcana*; *Speciosu*m. *Pak. J. Pharma. Sci*., **23(1)** : 29-34.

Pilz, D., L. Norvell, E. Danell and R. Molina (2003). Ecology and management of commercially harvested Chanterelle mushrooms. Gen. Tech. Rep. PNW-GTR-576. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. pp. 1-16.

Rahi, D. K., K. K. Shukla, R. C. Rajak and A. K. Pandey (2004). Mushrooms and their sustainable utilization. *Everyman's Sci*., **38(6)** : 357-365.

Singleton, V. L. and J. A. Rossi (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic*., **6** : 144-158.

Song, W. Y., K. H. Ku and J. H. Choi (2010). Effect of ethanol extracts from red pepper seeds on antioxidative defense system and oxidative stress in rats fed high-fat, high-cholesterol diet. *Nutr. Res. Pract*., **4(1)** : 11-15.

Stankovic, M. S. (2011). Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. *Kragujevac J. Svi*., **33** : 63-72.

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