PHYTOCHEMICAL, ANTIOXIDANT AND ANTIBACTERIAL POTENTIAL OF EXTRACTS OF CICHORIUM INTYBUS (CHICORY)

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ABSTRACT
The Kasni, Cichorium intybus (C. intybus) is well known traditional herb included in many systems of medicine like Ayurveda, Unani and Siddha. Keeping in view its medicinal value phytochemical, antioxidant and antibacterial analysis of extracts of leaves and roots of C. intybus was carried out. Phytochemical analysis revealed the presence of diterpenes, alkaloids, flavonoids, proteins, amino acids, saponins, phenols, phytoстерols and tannins in the extracts but glycosides, cardiac glycosides and carbohydrates were absent. Antioxidants were determined by total phenolic content (TPC) and DPPH free radical scavenging activity. Methanol extracts of leaves and roots exhibited maximum phenolic content and DPPH free radical scavenging activity followed by chloroform extracts and aqueous extracts of leaves and roots. As the concentration of extracts increases from 10µg/ml to 100µg/ml the scavenging effect also increases for all the extracts. The antibacterial activity against S. aureus, B. subtilis, E. coli and P. aeruginosa shows that methanol and chloroform extracts of leaves and roots had significant antibacterial activity with maximum zone of inhibition against E. coli. But aqueous extracts of leaves and roots exhibited highest antibacterial activity against P. aeruginosa. It is concluded from the results that C. intybus has potential source of natural antioxidants and can be used for the treatment of diseases caused by the test organisms. Due to good phytochemical, antioxidant and antibacterial composition, C. intybus would be valuable candidate in pharmaceutical formulations and play an important role in improving the human, livestock and poultry health by participating in the antioxidant defence system against endogenous free radicals and disease causing bacteria.

KEYWORDS: Alkaloids, Antibacterial, Free radicals, Hexane, Phenolic content.

1. INTRODUCTION
Plant derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube et al., 2008).

Higher and aromatics plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts. Biologically active compounds from natural sources have always been a great interest for scientists working on infectious diseases (Nandagopal and Kumari, 2007). Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. The most important of these bioactive compounds are alkaloids, flavonoids, tannins and phenolic compounds which are the important raw materials for drug production. A wide variety of these secondary metabolites (bioactive compounds) have been found in vitro to have antimicrobial properties for protection against aggressor agents, especially microorganisms (Silva and Junior, 2010; Bhalodia and Shukla, 2011).

Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity. Therefore, it is of great interest to carry out a screening of the plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterisation of their constituents (Mehmood et al., 2012). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Much work has been done on ethno-medicinal plants in India. In an effort to expand the spectrum of phytochemical, antioxidant and antibacterial agents from natural resources, Cichorium intybus (C. intybus) belonging to Asteraceae family has been selected in this study.

MATERIALS AND METHODS
Collection of Plant Material
The C. intybus plants were collected from agricultural fields to obtain roots and leaves and transferred to the
laboratory for further processing. Roots and leaves of C. intybus were separated manually with sharp knife, washed with distilled water and dried in hot air oven for one week. After one week, grinded into fine powder using an electric blender. The powder was then dried in oven at 40°C for 24 hours and stored in air tight jars for further analysis. The fine powdered plant parts were used for the preparation of extracts.

Preparation of Extracts
Water, chloroform and methanol were used as solvents for the preparation of extracts of roots and leaves. 25gram of powdered root and leaves were soaked separately in 100ml of each solvent and kept in a shaker for 3 days. After 3 days the mixtures were filtered through Whatman filter paper number1 and filtrates were stored in air tight bottles for further analysis.

Phytochemical Screening of Extracts
Extracts were tested for the presence of active principles such as diterpenes, alkaloids, flavonoids, proteins, free amino acids, glycosides, cardiac glycosides, carbohydrates, saponins, phenols, phytosterols and tannins following standard procedures (Tiwari et al., 2011).

Antioxidant Analysis
The C. intybus aqueous, chloroform and methanol extracts were further tested for presence of antioxidants by total phenolic content and DPPH free radical scavenging assay.

1. Total phenolic content (TPC)
The total phenolic content of the extract was determined by the Folin-Ciocalteu method. Briefly, 200μl of crude extract were made up to 3ml with distilled water, mixed thoroughly with 0.5ml of Folin-Ciocalteu reagent for 3 minutes, followed by the addition of 2ml of 20% (w/v) sodium carbonate. The mixture was allowed to stand for 60 minutes in dark, and absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per gram dry weight of sample. Each assay was carried out in triplicate (Baba and Malik, 2015).

2. DPPH radical scavenging assay
The antioxidant activity of the samples was assessed through their ability of scavenging 2, 2-diphenyl-1-picrylhydrazyl stable radicals (DPPH). The samples (from 10 to 250μg/ml) were mixed with 1ml of 90μM DPPH solution and made up with 95% methanol, to a final volume of 4ml. After incubation of 60 minutes at room temperature, the absorbance was recorded at 515nm. All samples were analysed in triplicates and average of three results was calculated. A negative control was prepared by mixing DPPH solution to 0.1ml Methanol. Percent radical scavenging concentration was calculated using the following formula:

\[
\text{Radical Scavenging (\%) } = 100 \times \left( \frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Blank}}} \right)
\]

Here, \( A_{\text{Blank}} \) is the absorbance of the control (containing all reagents except the test sample), and \( A_{\text{Sample}} \) is the absorbance of the test samples (Mehmood et al., 2012).

Determination of Antibacterial Activity
Two Gram positive bacteria - Staphylococcus aureus (MTCC 6810) and Bacillus subtilis (MTCC 1427) and two Gram negative - Escherichia coli (MTCC 443) and Pseudomonas aeruginosa (MTCC 2453) were chosen for evaluation of antibacterial activity of C. intybus extracts of leaves and roots.

Antibacterial activity of C. intybus extracts was tested by using Agar well diffusion technique on Muller Hinton agar (MHA) media. The presence of zone of inhibition (mm) indicated the antibacterial activity. Each extract of C. intybus was tested against the test organisms in triplicates along with media control and organism control plates (Zearaha et al., 2013).

Minimum Inhibitory Concentration (MIC)
The least concentration of the extract that is able to inhibit the growth of the bacteria was determined by the macro broth dilution method. To approximately 2ml of prepared nutrient broth in series of sterile and well labelled test tubes, 2 drops of the test bacteria (previously diluted to 0.5 McFarland turbidity standard) and 2 drops of different concentrations (50, 100, 150, 200 and 250 500ug/ml) of extracts were added. The same procedure was repeated on the test organisms using the different concentrations (50, 100, 150, 200 and 250 500ug/ml) of the antibiotic (chlorompenicol), a test tube containing nutrient broth only seeded with the test organism was used as control. The tubes were then properly corked and incubated at 37°C for 24hours. The MIC was read and recorded as the least concentration of the extract that shows no visible bacterial growth (Ugoh and Haruna, 2013).

RESULTS AND DISCUSSION
The present study was performed to find out the phytochemical constituents, antioxidant and antibacterial activity of Chicorium intybus leaves and roots. For this purpose aqueous, chloroform and methanol extracts of C. intybus parts were prepared.

Phytochemical Screening of Extracts
Preliminary phytochemical analysis revealed that important phytochemical components such as diterpenes, alkaloids, flavonoids, proteins, amino acids, saponins, phenols, phytosterols and tannins were found to be present in almost all the extracts of leaves and roots of C. intybus but glycosides, cardiac glycosides, carbohydrates were absent in all the extracts (Table 1). The presence of flavonoids and tannins in C. intybus extract are in agreement with previous reports (Mehmood et al., 2012;
Nandagopal and Ranjitha, 2007; Muthusamy et al. 2008).

Tannins, the high molecular weight polyphenolic compounds found naturally in many plants and have been found to play a protective role in plants against micro-organisms, unfavourable climatic conditions and damage by animals. On the other hand, they can form multiple hydrogen bonds with carboxylic groups of dietary proteins and proteolytic enzymes in the gastrointestinal tract which leads to reduced digestibility of proteins and finally the retardation of animal growth. Saponins are the glycosidic compounds found in most of the plants, possess a bitter taste and foaming properties. Saponins have been found to possess anticarcinogenic and antifungal activity (Abbas et al., 2015).

The flavonoids and phenolic acids are known to possess antioxidant activities due to the presence of hydroxyl groups in their structures and their contribution to defence system against the oxidative damage due to endogenous free radicals is extremely important (Saggu et al., 2014). Phenolics or polyphenols are secondary plant metabolites that are ubiquitously present in plants and their products. Many of them have been shown to contain high levels of antioxidant activities (Razali et al., 2008). Due to their redox properties these compounds contribute to the overall antioxidant activities of plants. Usually, the mechanisms of their antioxidant activity are neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals (Li et al., 2009).

Antioxidant Activity

1. Total phenolic content (TPC)

Results shows that C. intybus is rich in phenolic content and TPC were found in the range of 23.4 to 62.5 mg GAE/100 gm dry weight when calculated by using standard curve of gallic acid. Methanol extract of leaves contain highest TPC (62.5 mg GAE/100 gm dry weight) and aqueous extract of roots contain lowest TPC (23.4 mg GAE/100 gm dry weight). TPC in chloroform extracts of leaves and roots was found to 48.8 and 41.4 mg GAE/100 gm dry weight respectively. Aqueous extracts of leaves and roots contain 30.2 and 23.4 mg GAE/100 gm dry weight TPC respectively (Table 2).

Table 2. Total Phenolic Content in Extracts of C. intybus

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extract</th>
<th>TPC (mg GAE/100g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Aqueous Extract of Leaves</td>
<td>30.2</td>
</tr>
<tr>
<td>2.</td>
<td>Aqueous Extract of Roots</td>
<td>23.4</td>
</tr>
<tr>
<td>3.</td>
<td>Chloroform Extracts of Leaves</td>
<td>48.8</td>
</tr>
<tr>
<td>4.</td>
<td>Chloroform Extracts of Roots</td>
<td>41.4</td>
</tr>
<tr>
<td>5.</td>
<td>Methanol Extracts of Leaves</td>
<td>62.5</td>
</tr>
<tr>
<td>6.</td>
<td>Methanol Extracts of Roots</td>
<td>54.6</td>
</tr>
</tbody>
</table>

It was observed that the methanol extract had more antioxidant activity than other extracts studied. The amounts of TPC from C. intybus leaves and roots in different solvent systems were in the ranges 30.2-62.5 GAE (mg /100 gm of dry plant matter) and 23.4-54.6 GAE (mg/100 g of dry plant matter), respectively (Table 2). Methanol extract of the C. intybus leaves and roots showed the highest TPC respectively. These differences in the amount of TPC may be due to varied efficiency of the extracting solvents to dissolve endogenous compounds. The ability of different solvents to extracts TPC was of the order: methanol > chloroform > aqueous (Table 2 and Fig. 1).
Total Phenolic Content (TPC) in Extracts of *C. intybus*

Methanol is most efficient and widely used to extract anti-oxidative components including phenolic acids and other phenolic components. Although chloroform and water also extracted reasonable amounts of TPC, however, due to comparatively lower polarity, were less effective.

2. DPPH free radical scavenging assay

Results shows that as the concentration of phenolic compounds or degree of hydroxylation of the phenolic compounds increases, DPPH scavenging activity also increases, hence does the antioxidant activity. *C. intybus* leaves and roots showed maximum DPPH free radical scavenging activity in methanol (78.62% and 71.88% respectively) followed by chloroform (68.54% and 64.21% respectively) and water (62.40% and 53.36% respectively) (Table 3 and Fig. 2).

### Table 3. DPPH Free Radical Scavenging Activity of Extracts of *C. intybus*

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Scavenging Activity (%) of Aqueous Extract</th>
<th>Scavenging Activity (%) of Chloroform Extract</th>
<th>Scavenging Activity (%) of Methanol Extract</th>
<th>Scavenging Activity (%) of Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Roots</td>
<td>Leaves</td>
<td>Roots</td>
</tr>
<tr>
<td>10</td>
<td>6.8</td>
<td>5.9</td>
<td>7.9</td>
<td>6.8</td>
</tr>
<tr>
<td>20</td>
<td>10.60</td>
<td>9.69</td>
<td>12.05</td>
<td>10.45</td>
</tr>
<tr>
<td>40</td>
<td>21.66</td>
<td>18.45</td>
<td>21.39</td>
<td>20.09</td>
</tr>
<tr>
<td>60</td>
<td>29.48</td>
<td>22.79</td>
<td>32.84</td>
<td>28.76</td>
</tr>
<tr>
<td>80</td>
<td>48.22</td>
<td>39.61</td>
<td>55.67</td>
<td>50.17</td>
</tr>
<tr>
<td>100</td>
<td>62.40</td>
<td>53.36</td>
<td>68.54</td>
<td>64.21</td>
</tr>
</tbody>
</table>

The scavenging effect of different solvent extracts on the DPPH radical decreased in the order of methanol> chloroform> water. Among these extracts, the methanol extract showed maximum antioxidant activity followed by chloroform extract and aqueous extract (Table 3 and Fig. 2). The methanol extracts also had higher total polyphenolic content than the chloroform extracts (Leaves: 62.05 and 48.8mg/g extract respectively; Roots: 54.6 and 41.4 mg/g extract respectively) (Table 2).

Fig. 2. Comparison of Scavenging Effect (%) of Leaves and Roots of *C. Intybus*

Leaves of *C. intybus* are found to be good source of phenolic compounds. Due to high phenolic content, comparatively good reducing power and DPPH radical scavenging capacity was found in leaves. All the extracts of leaves were found to be rich in high phenolic content as compared to root extracts which may be responsible for its observed antioxidant activity. To evaluate the free radical scavenging effect of plant extracts, DPPH test is a widely used method. In this method methanolic DPPH solution is reduced in the presence of antioxidant to form non radical DPPH-H. The degree of discoloration shows the scavenging potential of the extract. The extracts had dose dependent activity, i.e. DPPH scavenging activity increased proportionately to the increase in concentration of the extracts (Table 3 and Fig. 2).

Due to the presence of phenolic compounds *C. intybus* has been found to have great medicinal value. The results show that all of the studied parts of *C. intybus* are good source of phenolic compounds. Leaves found to possess comparatively good DPPH radical scavenging capacity because of high total phenolic content. The root has been found to show comparatively low antioxidant activities due to low phenolic content (Table 2 and 3).

Antibacterial Activity and Minimum Inhibitory Concentration (MIC)

Both polar as well as non-polar solvents were used in this study for the extraction of active components from the leaves and roots of *C. intybus*. The results showed that all the solvent extracts possesses antibacterial
activity against the tested Gram positive (S. aureus and B. subtilis) and Gram negative (E. coli and P. aeruginosa) bacteria. Methanol extracts of leaves and roots showed pronounced inhibition against all the tested organisms, the maximum inhibition was observed on E. coli (ZOI 13.6 and 12.0mm respectively) and minimum on S. aureus (ZOI 10.4 and 8.2mm respectively) (Table 4).

Table 4. Zone of Inhibition (ZOI) of C. intybus Extracts against Test Bacteria

<table>
<thead>
<tr>
<th>Test Bacteria</th>
<th>ZOI (mm) of Leaves Extracts</th>
<th>ZOI (mm) of Roots Extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous</td>
<td>Chloroform</td>
</tr>
<tr>
<td>S. aureus</td>
<td>5.6</td>
<td>9.2</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>8.4</td>
<td>9.6</td>
</tr>
<tr>
<td>E. coli</td>
<td>9.2</td>
<td>12.4</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>9.8</td>
<td>11</td>
</tr>
</tbody>
</table>

Aqueous extracts of leaves and roots of C. intybus produced maximum inhibition (ZOI 9.8 and 9.0mm respectively) to the growth of P. aeruginosa whereas chloroform extracts of leaves and roots showed maximum inhibitory activity (ZOI 12.4 and 11.6mm respectively) against E. coli. It was found that both aqueous as well as organic extracts of the leaves and roots were successful in inhibiting the bacteria (Table 4 and Fig. 3).

Fig. 3. Comparison of Inhibition Zones (ZOI) of Extracts of Leaves and Roots of C. Intybus

Table 5. MIC (µg/ml) of C. intybus Extracts Against Test Bacteria

<table>
<thead>
<tr>
<th>Test Bacteria</th>
<th>MIC (µg/ml) of Aqueous Extract</th>
<th>MIC (µg/ml) of Chloroform Extract</th>
<th>MIC (µg/ml) of Methanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Roots</td>
<td>Leaves</td>
</tr>
<tr>
<td>S. aureus</td>
<td>250</td>
<td>250</td>
<td>150</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>200</td>
<td>250</td>
<td>150</td>
</tr>
<tr>
<td>E. coli</td>
<td>150</td>
<td>200</td>
<td>50</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>150</td>
<td>150</td>
<td>100</td>
</tr>
</tbody>
</table>

The results of MIC indicated that methanol extracts of leaves and roots showed strong activity against E. coli evident from the lowest MIC values (50µg/ml) and least activity was against S. aureus with the smallest inhibition zones (10.4 and 8.2mm) and the highest MIC values (150 and 200µg/ml), respectively. Chloroform extracts of leaves and roots showed good activity against E. coli with inhibition zones of 12.4mm and 11.6mm and the lowest MIC values (50 and 100µg/ml), respectively. Least activity was exhibited against S. aureus, with the smallest inhibition zones (9.2 and 6.6 mm respectively) and the highest MIC values (50 and 100µg/ml respectively). Aqueous extracts of leaves and roots of C. intybus showed strong activity against P. aeruginosa (ZOI 9.8 and 9.0mm respectively) and the lowest MIC values (150µg/ml). Least activity was exhibited against S. aureus with inhibition zones 5.6 and 5.2mm respectively and the highest MIC values (250µg/ml) (Table 4 and 5). In general, the results indicated that plant extracts showed good antibacterial activity.

Antimicrobial effect of chicory leaf extract with different solvents on S. aureus and E. coli was studied by Khazadhe et al.. S. aureus and E. coli antibiograms with different solvents had no significant difference between groups and no inhibition zone observed around different extract disks and control disks on Mueller-Hinton agar culture. They couldn’t find any chicory leaves extract antibacterial effect against S. aureus and E. coli (Khazadhe et al., 2014).

Ethyl acetate extract of chicory root was tested for antibacterial and anti-fungal properties. Fractionation by column chromatography of ethyl acetate extracted root powder contains the compound, inhibiting both Gram positive and Gram negative bacteria and was found to be bacteriostatic rather than bacteriocidal. The effect of chicory root extract has more bacteriostatic effect on Gram Positive bacteria than Gram negative bacteria as MIC value is more in case of Gram negative bacteria than Gram positive bacteria. The ethyl acetate fraction of root which is obtained by silica gel column
chromatography has also antifungal activity as it inhibits growth of yeast and moulds (Koner et al. 2011). Extract of C. intybus L. had mild antibacterial effect against E. coli and S. aureus and it was shown that there is a synergistic antibacterial effect between the respective medicinal plants with tetracycline, chloramphenicol and ciprofloxacin (Aqil and Ahmad, 2007). In a research conducted by Ghaderi et al (2012) C. intybus L. had no antibacterial effect on Streptococcus pyogen, Staphylococcus aureus and Enterococcus. They studied comparison of antibacterial effect of C. intybus L. with vancomycin, ceftriaxone, ciprofloxacin and penicillin (Ghaderi et al., 2012). In vitro antibacterial activity of C. intybus against some pathogenic bacteria was studied. It was reported that plant fractions under study have great potential as antibacterial compound against E. coli and P. aeruginosa and they can be used in the treatment of infectious diseases caused by above resistant microorganisms (Verma et al. 2013).

CONCLUSIONS
In conclusion, all tested parts of C. intybus contain considerable amounts of phytochemicals and are good source of antioxidants. However, methanol extracts of C. intybus leaves possess comparatively higher amounts of phytochemicals, antioxidants and antibacterial compounds and also show high free radical scavenging capacity. Results contributed that, chicory contains higher concentration of organic solvent soluble compounds that may be responsible for exhibiting higher antioxidant and antibacterial activity. It may be suggested that C. intybus leaves, would play an important role in antioxidant defense system against endogenous free radicals because of their good antibacterial and antioxidant composition and thus improving the human health. The results confirmed the therapeutic potency of the plant scientifically and support the effectiveness of C. intybus to treat infectious disease locally as well as traditionally. The results also form a good basis for utilization of this plant for further pharmacological and toxicity research. The further need is to study the different organic fractions of this plant to draw complete picture of its potential as antioxidant and antibacterial agent.

CONFLICT OF INTEREST
Authors have no conflict of interests in this study.

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