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Bio chemical, phytochemical and antibacterial analysis of *Chara braunii* c.c. gmelin

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Abstract
The study focused on the estimation of biochemicals, primary phytochemicals analysis and antibacterial activity of *Chara braunii* c.c.gmelin Alga. Biochemical estimation revealed the presence of Carbohydrate (19.27±0.37), Protein (34.00±0.58), Chlorophyll (1.34±0.00), Amino acid (30.67±0.67) and Phenol (08.63±0.09). Alkaloids, Tannins, Lignins, Flavonoids, Phenolic compounds, Steroids and Anthocyanins phytochemicals are identified. *Chara braunii* inhibited the *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Keywords: antibacterial, biochemical, *Chara*, phytochemical, Talakona

1. Introduction
Algae are used as food, feed and medicine from immemorial time. The primary metabolites are essential for the growth and development of algae, whereas secondary metabolites have the defence against the diseases and grazing animals (Morris *et al*, 1995) [14]. The phytochemicals are non-nutritive chemicals in the plants, which have the capacity to protect the human against diseases (Kumar *et al*, 2009) [11]. Saponins, terpenoids, tannins, steroids, alkaloids and flavonoids act as anti-inflammatory agents (Latha *et al*, 1998) [12]. Phytochemical screening is important step which leads to the isolation and separation of secondary metabolites, which are used in medicine.
Charophyceae are macroscopic, grow erect with rhizoid, 2 to 60 cm height, stem like branches and whorls of secondary branches. They habitat in the slow streams or bottom of lakes. Chara grow in the semi hard water, beside the whorl of leaves, the stem nodes shows stipulodes and leaves are smooth.

2. Materials and Methods
2.1 Collection and identification of algae material
Chara braunii c.c. gmelin collected from the fresh water streams of Talakona region, Chittoor district, Andhra Pradesh. The samples were isolated from other associated algae, cleaned thoroughly with the water.

2.2 Biochemical analysis
The whole fresh material was used for quantitative estimation of Carbohydrates (Anthrone method), Protein (Lowry's method), Chlorophyll (Acetone method), Amino acid (Ninhydrin test) and Phenol (Folin-Ciocalteau reagent test).

2.3 Phytochemical analysis
The collected alga *Chara braunii* was cultured in *in vivo* condition, washed and dried under room temp

erature for one week and grounded. The plant powder was stored in air tight container for further analysis of Alkaloids, Flavonoids, Phenolic compounds, Steroids, Saponins, Tannins, Anthocyanin, Lignin, Glycosides in three different solvents (Aqueous, Alcohol and Methanol) by Harborne method (Harborne, 1984) [6].

2.4 Anti-bacterial activity
Anti-bacterial activity was conducted with 3 different extracts (Aqueous, Alcohol and Methanol) for *Hydrodictyon reticulatum, Nitella hyalina* and *Chara braunii* determined by the agar well method according to the National committee for clinical labotary standards. Bacterial culture inoculated in nutrient broth agar medium and plates were punched for well. The wells were filled with 100µl 3 different extracts, incubated for 24 hours at 30 ^{0}C temperature. After completion of incubation period the inhibition zones were measured in mm. The bacterial strains *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsella pneumoniae* were collected from the department of Microbiology, Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati.

Fig 1

3. Results and Discussion
3.1 Identification of *Chara braunii* c.c. gmelin
Plant body is 30 cm long, 620 µm wide and 6-9 whorls branch lets, incurved, terminal cell with 3 projection. Sex

organs present at first node, oogonium 750 µm length and 480 µm broad, Corona 130 µm length and Antheridium 350 µm in diameter.

3.2 *Chara braunii* c.c. gmelin Biochemical Analysis

The biochemical estimation of *Chara braunii* shows presences of Carbohydrate (19.27±0.37), Protein (34.00±0.58), Chllorophyll (1.34±0.00), Amino acid (30.67±0.67) and Phenol (08.63±0.09). Biochemical deals with the chemical and physical properties of molecule and living organisms. The presence of high proteins content in the algae used dietary food supplement to the human beings and animals' feed. Chlorophylls have the antioxidant and antimutagenic properties.

Table 1: Biochemical analysis of *Chara braunii* c.c. gmelin

S. No	Name of the Biochemical	Biochemical content (mg)
1.	Carbohydrates	19.27±0.37
2.	Proteins	34.00±0.58
3.	Chlorophylls	1.34±0.00
4.	Amino acids	30.67±0.67
5.	Phenols	08.63±0.09

3.3 Phytochemical Analysis

The phytochemical analysis of *Chara braunii* aqueous extract revealed the presence ofAlkaloids, Lignins, Flavonoids, Tannins, partial presence of Phenolic compounds, Steroids and Anthocyanins, Glycosides, Saponins are absent. Alcohol extract shows presence of Alkaloids, Tannins, Lignins, partial presence of Flavonoids, Phenolic compounds, Steroids, Anthocyanins and Saponins, Glycosides are absent. Methanol extract shows presence of Alkaloids, Lignins, partial presence of Flavonoids, Phenolic compounds, Steroids, Glycosides and Saponins, Anthocyanins, Tannins are absent. Among the three extracts alcohol extract shows the high number of phytochemicals, followed by the aqueous and methanol extracts.

Table 2: Phytochemical analysis of *Chara braunii* c.c. gmelin

S. No	Name of the Phytochemical	Name of the test	Aqueous	Alcohol	Methanol
1.	Alkaloids	Mayer's test	++	++	++
		Wagner's test	++	++	++
2.	Flavonoids	Shinoda's test	+	-	+
		Ferric chloride test	++	+	+
3.	Phenolic compounds	Phenols test	+	+	+
		Ellagic acid test	-	-	-
4.	Steroids	Salkowski test	-	+	+
		Libermann's test	+	+	+
5.	Saponins	Saponins test	-	-	-
6.	Tannins	Gelatin test	++	++	-
		Ferric chloride test	+	+	-
7.	Anthocyanin	Anthocyanin test	-	+	-
8.	Lignin	Lignin's test	++	++	+
		Labat test	++	++	++
9.	Glycosides	Kilerkillani test	-	-	+

3.4 Anti-bacterial activity

Chara braunii alcohol extract highly inhibited the *Escherichia coli* (8.77 mm) followed *by Staphylococcus aureus* (8.4mm), *Pseudomonas aeruginosa* (*7.43* mm) and *Klebsiella pneumoniae* (6.2 mm*). Chara braunii* methanol extract highly inhibited the *Escherichia coli* (7.03 mm) followed *by Staphylococcus aureus* (6.87 mm), *Pseudomonas aeruginosa* (5.73 mm) and *Klebsiella pneumoniae* (4.6 mm*).*

Chara braunii aqueous extract highly inhibited the *Escherichia coli* (5.27 mm), followed by *Pseudomonas aeruginosa* (4.5 mm), *Staphylococcus aureus (*4.43 mm) and *Klebsiella pneumoniae* (3.2 mm*).* Among three extracts, alcohol extract highly inhibited both gram positive and negative bacteria compare to the methanol and aqueous extracts.

The phytochemicals have the chemo preventive properties like antioxidant, anti-carcinogenic, so they have the importance in the pharmacognosy (Huang, 2010) [7]. Phytochemicals synthesised in plants defend against pathogenic microbes, so they act as antimicrobial.

Algae have medicinal importance like antibiotic, antiviral, antioxidant, anti-cancer, anti-inflammatory, anti-cardiovascular, nutraceutical activity (Jai Prakash Keshri, 2012) [8]. Manikanta *et al,* (2018) [13] reported similar results in the genus *Chara* presence of shows antibacterial against both positive and negative bacteria.

Table 3: Anti-bacterial activity of *Chara braunii* c.c. gmelin

S. No	Name of the extract	*Staphylococcus aureus* (mm)	*Escheric a coli* (mm)	*Pseudomonas aeruginosa* (mm)	*Klebsiella pneumoniae* (mm)
1.	Aqueous Extract	4.43±012	5.27±0.07	4.50±029	3.27±0.18
2.	Ethanol Extract	8.40±0.20	8.77±0.15	7.43±0.23	6.27±0.13
3.	Methanol extract	6.87±0.07	7.03±0.09	5.73±0.18	4.60±0.20

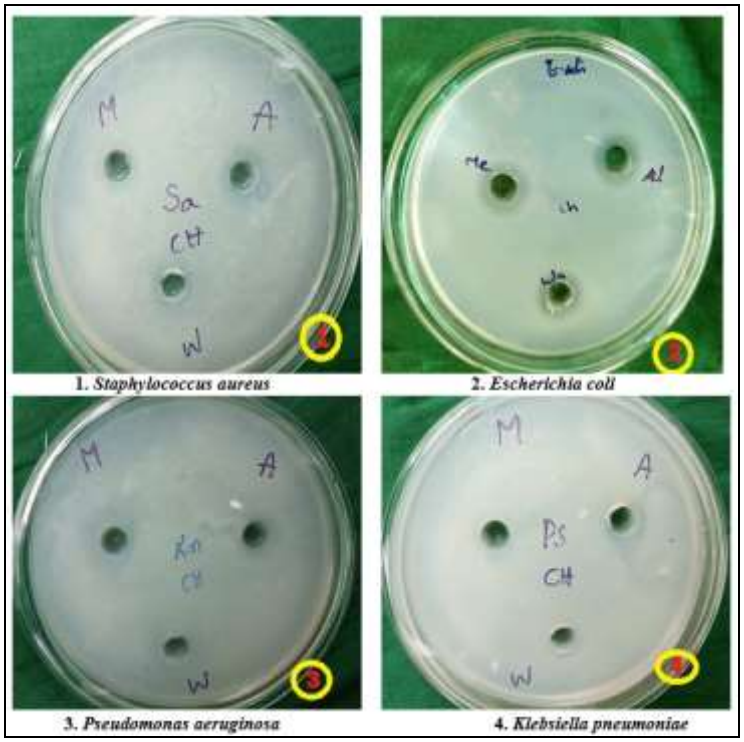

Fig 2: Anti-bacterial activity of *Chara braunii* c.c. gmelin

4. Conclusion

Based on biochemical estimation *Chara braunii* c.c.gmelin has the importance in food, feed and Pharmaceutical industries. The Alkaloids, Lignins, Flavonoids, Tannins, Phenolic compounds and Steroids phytochemicals are identified in *Chara braunii* c.c.gmelin. Presence of these phytochemicals shows antibacterial activity on both gram positive and negative bacteria. Among them *Chara braunii* c.c. gmelin highly inhibited the *Escherichia coli bacteria.*

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