SCREENING OF PHYTOCHEMICALS & BIOACTIVE ANTIBACTERIAL ACTIVITY IN SPIROGYRA SP.

Prarthana J¹ and Maruthi KR².

1. Department of P.G. Studies & Research in Biotechnology S.D.M. (Autonomous) College, Ujire-574 240 Karnataka, India.
2. Research Supervisor, PRIST University, Tamilnadu 613 403.

Recived: 12 May 2017  
Final Accepted: 14 June 2017  
Published: July 2017

Key words:-
Algae, Spirogyra sp., phytochemical, antibacterial activity, GC-MS, bioactive.

Abstract
Algae are very important component of aquatic ecosystem, known for producing several biologically active compounds with antiviral, antibacterial, antifungal, and anticancer activities. In the present investigation, the effect of different solvents, including methanol, acetone, petroleum ether, benzene, chloroform, hexane, diethyl ether, ethanol, ethyl acetate were done on Spirogyra sp. Dried algal powder after identification were cold extracted & screened for glycosides, alkaloids, saponins, flavonoids, tannins, terpenoids, phenolics, anthraquinones, cardiac glycosides, etc. The extracts showing good phenolic content were tested for antibacterial activity against pure cultures of fish pathogens namely Aeromonashydrophila. The GC-MS analysis of the extracts revealed the presence of several antimicrobial bioactive constituents in the extracts.

Introduction:-
Aquatic organisms are a rich source of structurally novel & biologically active metabolites[8]. Many microscopic forms of algae are known to grow in fresh water as slimy ‘blanket weed’. One such familiar algae is Spirogyra, a filamentous algae, its cells form each filament consists of an extensive chain of identical cells. Spirogyra is the most common genus of Zygmemataceae and member of the freshwater algae. It exhibits the greatest diversity of the 12 to 13 genera recognized in this family of green algae (Transeau 1951, Kadolubowska 1972).

Seasonal variation in pH, temperature & dissolved oxygen play an important role in the multiplication of pathogens leading to disease in fish [73]. Aeromonashydrophila is an opportunistic pathogen known to cause disease during stressed condition. Symptoms of the disease may range from sudden death to lack of appetite, swimming abnormalities, pale gills & skin ulceration. Current treatment involves use of antibiotics such as Tetramycin®, an oxytetracycline, and Remet-30®. Potential problems associated with antibiotic include inadequate dosage, overdosing, drug resistance by bacteria[74]

The use of algae for therapeutic purpose has a long history & systemic examination of algae for biologically active substances. The aqueous & solvent extracts from algae were tested against gram positive & gram negative bacteria [7-10]. The first investigation of the antibiotic activity of algae was carried out by (Pratt et al., 1944). Evidence of phytochemical and pharmacological studies on algae is available in the literature with special references to terpenoids and steroids (Parameswaran et al. 1944; Patterson et al 1968). Secondary and primary metabolites...
produced by these organisms may be potential bioactive compounds of interest in pharmaceutical industry. There are a number of reports on the evaluation of antioxidant activity in microalgae and cyanobacteria belonging to the genera *Botryococcus* (Rao et al. 2006), *Chlorella* (Wu et al. 2005; Goh et al. 2010), *Dunaliella* (Herrero et al. 2006), *Nostoc* (Li et al. 2007), *Phaeodactylum* (Guzman et al. 2001), *Spirulina* (Miranda et al. 1998; Jaime et al. 2005; Mendiola et al. 2007), *Haematococcus* (Cerón et al. 2007) and *Chaetoceros* (Goh et al. 2010). These studies concluded that several microalgal genera contain potent antioxidants, both from lipophilic and hydrophilic nature. Justella et al. (2011) proved antimicrobial property of ethanolic extracts of *Spirogyra grantiana* against *E. coli*. Antimicrobial & antioxidants activity of *Euglena tuba*, *Oscillatoria agardhii & Anabena sphaerica*, *Cosmarium sp.*, *Chlorococcum humicola* (Dipankar et al. 2014; Abd El-Aty et al. 2014; Challouf et al. 2012; Bhagavathy et al. 2011). In the present study dried algal extracts were dissolved in different solvents with the increasing order of polarity, different extracts were tested for the presence of chemical constituents. The extracts showing good phenolic content were estimated & analyzed for antimicrobial activity against fish pathogens *Aeromonas hydrophila*. GC-MS analysis of the *Spirogyra* Diethyl ether, Acetone & Ethyl acetate extracts reported several bioactive components with antimicrobial property.

**Material and method:**

**Sample preparation & extraction:**

Samples were collected from Arasinamakki, near Shishila. Dakshinakannada, India. Algae samples were cleaned all epiphytes and necrotic parts were removed. Samples were rinsed with sterile water to remove any associated debris. Sample was kept under sunshade for 7 days. After drying the sample, it was ground thoroughly to powder form. The powdered samples were extracted with at room temperature with different solvents. The extraction is carried out for 10 days, after that extracts were filtered, the filtrate is dried using rot evaporator and concentration is determined & subjected to analysis. (Gonzalez del valet.al, 2001.)

**Phytochemical Analysis:**

The extracts were subjected to phytochemical tests for presence of following biomolecules by using the standard qualitative procedures as described in literature [15].

1. Test for Glycosides: 10 ml of 50% H2SO4 was added to the 1 ml of extract in a boiling tube. The mixture was heated in boiling water bath for 5 min. 10 ml of Fehling’s solution (5 ml of each solution A and B) was added and boiled. A brick red precipitate indicated the presence of glycosides.

2. Test for Alkaloids: 1 ml of 1% HCl was added to the 3 ml of extract in a test tube and was treated with few drops of Meyer’s reagent. A creamy white precipitate indicated the presence of alkaloids.

3. Test for saponins: 5 ml of extract was shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicated the presence of saponins.

4. Test for flavonoids: A few drops of 1% NH3 solution was added to the 2 ml of extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

5. Test for tannins: To 0.5 ml of extract solution, 1 ml of distilled water and 1-2 drops of ferric chloride solution were added and observed for brownish green or blue black coloration.

6. Test for terpenoids: 5 ml of extract was mixed with 2 ml of CH3CN in a test tube. 3 ml of concentrated H2SO4 was carefully added along the wall of the test tube to form a layer. An interface with a reddish brown coloration was confirmed the presence of terpenoids.

7. Test for cardiac glycosides: 5 ml of extract was mixed with 2 ml of glacial acetic acid containing 1 drop of FeCl3. The above mixture was carefully added to the 1 ml of concentrated H2SO4. Presence of cardiac glycosides was detected by the formation of a brown ring.

8. Test for phlobatannins: 10 ml of extract was boiled with 1% HCl in a boiling tube. Deposition of a red precipitate indicated the presence of phlobatannins.

9. Test for Anthraquinones: Extract was mixed well with benzene, and then half of its own volume of 10% ammonia solution was added. Presence of a pink, red or violet coloration in the ammonial phase indicated the anthraquinones.

10. Test for Phenols: 3 mL of 10% lead acetate solution were added to 5 mL of plant extract. A bulky white precipitates indicated the presence of phenols.

1146
Estimation of Phenolic Content:-
The amount of total phenolics[1] in methanol extract was determined with Folin–Ciocalteu reagent according to the method of Singleton and Rossi with Gallic acid as the standard [23]. Briefly standard stock solution of 10 mg/10 ml of gallic acid was prepared in distilled water. From this, various concentrations ranging from 200-1000 μg / ml were prepared. To this 1 ml Folin and Ciacalteau reagents (1:2 with water) was added and kept at room temperature for 5 min and then 1 ml of 7% sodium carbonate solution was added to the reaction mixture and incubated at room temperature for 90 minutes. The colour developed was read at 750 nm. A 100 μl of each extract of sample was mixed with the same reagents. Gallic acid was used as the reference standard and the results are expressed as milligram gallic acid equivalent (mg / g dry weight of Spirogyra sp).

Antibacterial Activity:-
Pure cultures of Aeromonas hydrophila, was used as test micro-organisms. Different solvent extracts were checked for antibacterial activity against the lawn cultures by agar well diffusion method. In each respective solvent is chosen as in the form of control.

Gas chromatography and mass spectrometry Analysis:-
Gas chromatography–mass spectrometry (GC–MS) analysis was performed using an BR-5MS(5%Diphenyl/95% Dimethyl poly siloxane)capillary column (length 30 m × diameter 0.25 mm × film thickness 0.25 μm) with helium at 1 ml for 1 min as a carrier gas. The mass spectrometer was operated in the electron impact (El) mode at 70 eV in the scan range of 50–500 m/z. The split ratio was adjusted to 1:10, and the injected volume was 2 μl. The injector temperature was 280 °C, and the oven temperature was kept at 110 °C for 3.5 min, rose to 280 °C at 5 °C min⁻¹ (total run time 37.50 min). Peak identification of crude Spirogyra extracts were performed by comparison with retention times of standards, and the mass spectra obtained were compared with those available in NIST libraries (NIST 11 – Mass Spectral Library, 2011 version) with an acceptance criterion of a match above a critical factor of 80% according to Srinivasan et al 2016

Results and Discussion:-

Phytochemical Analysis:-
Important phytochemicals, such as alkaloids, triterpenoids, steroids, tannin, saponin, coumarins, terpenoids, quinine, phytosteroids, phlobatannins and flavonoids were screened for their presence and presented in Table 1.

Table 1:- Phytochemical constituents of Spirogyra extracts.

| Spirogyra sp. | Ethanol Extract | Aceton. Extract | Methanol Extract | DiEthylEther. Extract | Benzen. Extract | Chloroform Extract | Hexane. Extract | Petroleum Ether Extract | Ethyl Acetate Extract |
|--------------|-----------------|-----------------|-----------------|-----------------------|----------------|--------------------|----------------|--------------------------|----------------------|
| Glycosides   | --              | --              | --              | --                    | --             | --                 | --              | --                       | --                   |
| Alkaloids:   | --              | --              | --              | --                    | ++             | ++                 | --              | ++                       | --                   |
| Spooning     | ++              | ++              | ++              | ++                    | ++             | ++                 | --              | --                       | --                   |
| Flavonoids   | --              | ++              | ++              | ++                    | --             | --                 | --              | ++                       | ++                   |
| Tannins      | --              | ++              | ++              | ++                    | ++             | ++                 | --              | ++                       | ++                   |
| Terpenoids   | --              | ++              | ++              | ++                    | ++             | ++                 | --              | ++                       | ++                   |
| cardiac glycosides | ++              | ++              | ++              | ++                    | ++             | ++                 | --              | ++                       | ++                   |
| Phlobatannins| --              | --              | --              | --                    | --             | --                 | --              | --                       | --                   |
| Anthraquinones | --            | --              | --              | --                    | --             | --                 | --              | --                       | --                   |
| Phenols      | ++              | ++              | ++              | ++                    | ++             | ++                 | --              | ++                       | ++                   |
| Sterols      | --              | ++              | --              | --                    | ++             | ++                 | --              | ++                       | ++                   |
| Resins       | --              | --              | --              | --                    | --             | --                 | --              | --                       | --                   |

++ Copiously present, + moderately present, - absent
Estimation of Phenolic content:-
Diethyl ether, Acetone & Ethyl acetate spirogyra extracts shown positive for tannins, flavonoids, terpenoids are analyzed. Highest phenolic content shown in ethyl acetate extract Table 2

| Sl.No | StdConc | OD 750nm | Sample extract(0.1ml) | OD at 750nm |
|-------|---------|----------|-----------------------|-------------|
| 1     | 0.2mg   | 0.24     | Acetone               | 0.63        |
| 2     | 0.4     | 0.41     | DEE                   | 0.04        |
| 3     | 0.6     | 0.90     | Eth Ace               | 1.09        |
| 4     | 0.8     | 1.22     |                       |             |
| 5     | 1.0     | 1.95     |                       |             |

Antibacterial activity:-
The antibacterial activity of Spirogyra extracts of Diethyl ether, Acetone & Ethyl acetate on Aeromonas hydrophila presented in Table 3. The agar well diffusion method was used to evaluate the antibacterial activity by measuring the zone of inhibition. Among three extracts Spirogyra ethyl acetate extract was found to be superior controlling growth of all three pathogens.

Table 3:- Antimicrobial activity of extracts depicted through zone of inhibition

| Sl.No | Sample Extract | Zone of inhibition in mm |
|-------|----------------|-------------------------|
|       |                | Aeromonashydrophila     |
| 1     |                | 10µl  25 µl  50 µl 100 µl C µl |
| 2     | DiEthyl Ether  | --  -- 10±0.11 11±0.32 08±0.43 |
| 3     | Acetone        | --  11±0.21 12±0.33 12±0.55 10±0.54 |
| 4     | Ethyl Acetate  | 15±0.56 18±0.41 23±0.33 26±0.57 13±0.62 |

Values are mean inhibition zone (mm) ± S.D of three replicate
MIC for Diethyl ether 5.54mg/ml, MIC for Acetone extract 3.75mg/ml, MIC Ethyl acetate 2.15mg/ml

GC-MS Analysis:-
The GC–MS analysis of the crude Spirogyra Diethyl ether extract, Table 4 revealed seven components of that the main chemical constituent was the Bus(2-ethylhexyl)phthalate Retention time(RT)23.94min 92.54% & n-Hexadecanoic acid RT 15.61 & 2.35%. Acetone extract, Table 5 revealed twenty four peaks of that main peaks were n-Hexadecanoic acid RT 15.82 & 29.02% , Bis(2-ethylhexyl)phthalate RT 23.88 & 21.60% , Tetradecanoic acid RT 13.13 & 16.46% & Octadecenoic acid RT 18.50 & 11.17 %. Ethylacetateextracts Table 6 showed thirty six peaks of that main peaks were Bis(2-ethylhexyl)phthalate RT 23.97 & 65.86%, n-Hexadecanoic acid RT 15.88 & 10.81%.

Table 4:- GC MS analysis of Spirogyra Diethyl ether extracts

| Name of the compound | RT | Mol.Wt | Peak Area% | Antimicrobial activity |
|----------------------|----|--------|------------|------------------------|
| ButylatedHydroxytoluene | 9.98 | 220 | 1.36 | Antioxidant, Antimutagenic, anticarcinogenic, inactivation of virus[28] |
| Methoprene           | 10.54 | 310 | 0.08 | Insectisidal[29] |
| Tetradecanoic acid   | 13.08 | 228 | 1.93 | Antibacterial & antifungal[30] |
| Dibutyl phthalate    | 15.55 | 278 | 1.56 | Antimicrobial, Disruptor of estrogen activity [31] |
| n-Hexadecanoic acid  | 15.62 | 256 | 2.35 | Antioxidant, hypcholesterolemic, anti-inflammatory, antibacterial[32] |
| Octadecanoic acid    | 18.44 | 284 | 0.19 | Cancer preventive Insectifuge* |
| Bis(2-ethylhexyl)phthalate | 23.94 | 390 | 92.54 | Antibacterial & antifungal[33] |
Table 5: GC MS analysis of Spirogyra Acetone extracts

| Name of the compound | RT  | Mol. Wt | Peak Area% | Activity                  |
|----------------------|-----|---------|------------|---------------------------|
| Octanoic acid        | 4.98| 144     | 0.02       | Fungicide,Pesticide,Candidicide* |
| Nonanoic acid        | 6.47| 158     | 0.32       | Herbicide[34]              |
| 2H-Pyran-2-one,tetrahydro-4-hydroxy-6-pentyl | 8.22 | 186 | 0.34 | Antimicrobial & anticancerous[35] |
| Undecylenic acid     | 9.09| 184     | 0.46       | Not reported              |
| 12-Hydroxydodecanoic acid | 9.63  | 216 | 0.84 | Antimicrobial, antioxidant, antiinflammatory, antitumor, anti-inflammatory[36] |
| 2(4H)-Benzofuran,5,6,7,7a-tetrahydro-4,4,7a-trimethyl | 10.43 | 180 | 1.50 | Antimicrobial[37] |
| Dodecanoic acid      | 10.71| 200     | 2.16       | Not reported              |
| Dihydrojasmonone     | 11.21| 166     | 0.99       | Antimicrobial & antifungal[38] |
| Azelaic acid         | 11.81| 188     | 1.43       | Antimicrobial[39]         |
| Tetradecanoic acid   | 13.13| 228     | 16.46      | Antimicrobial & anti-inflammatory[40] |
| 9,10-Dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol | 13.30  | 196 | 1.91 | Anticancerous[41] |
| 2-Pentadecanone,6,10,14-trimethyl | 14.07 | 268 | 2.26 | Repellent to arthropod[42] |
| Pentadecanoic acid   | 14.32| 242     | 2.55       | Antioxidant, antibacterial[43] |
| 2H-Pyran-2-one,tetrahydro-6-nonyl | 14.83 | 226 | 0.23 | Not reported            |
| n-Hexadecanoic acid  | 15.82| 256     | 29.02      | Antiinflammatory[44]      |
| 11,13-Dimethyl-12-tetradecen-1-ol acetate | 16.61  | 282 | 0.82 | Antioxidant & anticancerous activity[45] |
| Trans-13-Octadecenoic acid | 18.10 | 282 | 2.70 | Antimicrobial[46] |
| Octadecenoic acid    | 18.50| 284     | 11.17      | Free radical scavenging activity[47] |
| Erucic acid          | 19.07| 338     | 1.23       | Not reported              |
| z)-3,7,11-Trimethylododec-2-enoic acid, methyl ester | 19.93  | 254 | 0.34 | Not reported           |
| 7-Methyl-Z-tetradecen-1-ol acetate | 21.44  | 268 | 1.03 | Anticancer, anti-inflammatory, hepatoprotective[48] |
| Cis-11-EOcosenoic acid | 21.85 | 310 | 0.26 | Not reported           |
| Tricyclo[20.8.0.0(7,16)]triaccontane,1(22),7(16)-diepoxy | 22.74  | 444 | 0.37 | Antidiabetic type II[49] |
| Bis(2-ethylhexyl)phthalate | 23.88 | 390 | 21.60 | Antibacterial & antifungal[33] |

*Dr. Duke’s phytochemicals & ethanobotanical databases

Table 6: GC MS analysis of Spirogyra Ethyl acetate extracts

| Name of the compound | RT  | Mol. Wt | Peak Area% | Activity                                         |
|----------------------|-----|---------|------------|-------------------------------------------------|
| Nonanoic acid        | 6.93| 158     | 0.10       | Herbicide[34]                                   |
| Diethyl adipate      | 8.23| 202     | 0.34       | Antimicrobial anti inflammatory[50]             |
| Ethyl 5-methylhexonate | 8.51 | 158     | 0.00       |                                                 |
| Diethyl pimelate     | 9.65| 216     | 0.62       | Antioxidant & antimicrobial[51]                 |
| Nonanoic acid,9-oxo-,ethyl ester | 9.94  | 200 | 0.55 | Antioxidant, anti-inflammatory, anticancerous[52] |
| Ethyl hydrogen suberate | 10.63 | 202 | 0.16 | Antioxidant anti inflammatory[53]               |
| Diethyl suberate     | 10.96| 230     | 0.89       | Antifungal, antibacterial[54]                   |
| 3-Cyclopentylpropionic acid, ethyl ester | 11.12 | 170 | 0.18 | Antibacterial[55]                              |
| Chemical Formula                        | RT  | Molwt | Property                                |
|----------------------------------------|-----|-------|-----------------------------------------|
| Nonanedioic acid, monomethyl ester     | 11.87 | 202   | 0.66 Antitumour [56].                   |
| Nonanedioic acid, dimethyl ester       | 12.16 | 216   | 1.98 Antimicrobial, anti inflammatory[57]|
| 12-Oxododecanoic acid, ethyl ester     | 12.43 | 242   | 0.05 Antiseptic, antimicrobial, anti oxidant[58]|
| Thiazolo[3,2-a]pyrimidin-5-one, 7-methyl-2,3-dihydro- | 12.57 | 168   | 1.60 Antibacterial, antimicrobial [59] |
| Tetradecanoic acid                     | 13.17 | 228   | 4.87 No report                          |
| Decanedioic acid                       | 13.34 | 258   | 0.21 No report                          |
| Tetradecanoic acid, ethyl ester        | 13.45 | 256   | 1.92 Anticancerous[60]                  |
| 2-Pentadecanone, 6,10,14-trimethyl-    | 14.08 | 268   | 0.93 Not reported                       |
| Pentadecanoic acid                     | 14.23 | 242   | 0.27 Antimicrobial[61]                  |
| Ethyl 13-methyl-tetradecanoate         | 14.33 | 270   | 0.36 Antibacterial[62]                  |
| Dodecanedioic acid, dimethyl ester     | 14.59 | 258   | 0.26 Antibacterial, antidiabetic [63]   |
| Hexadecanoic acid, methyl ester        | 15.20 | 270   | 0.13 Antimicrobial anti inflammatory[64]|
| n-Hexadecanoic acid                   | 15.88 | 256   | 10.81 Antinflammatory[44]               |
| Hexadecanoic acid, ethyl ester         | 16.11 | 284   | 1.15 Antimicrobial, anti oxidant [58]   |
| Octadecanoic acid                      | 18.53 | 284   | 1.79 Free radical scavenging activity[47]|
| Octadecanoic acid, 17-methyl, methyl ester | 18.91 | 312   | 0.33 Antioxidant, antimicrobial[65]     |
| 3-Buten-2-one, 4-(3-hydroxy-6,6-dimethyl-2-methylencyclohexyl)- | 19.24 | 208   | 0.34 No report                         |
| Z)-3,7,11-Trimethyl decenoic acid methyl ester | 19.83 | 254   | 0.21 Insecticidal[66]                   |
| Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-pivalate | 20.76 | 498   | 0.68 Anticancerous, antiviral           |
| Octadecanoic acid, 10-oxo, methyl ester | 21.53 | 312   | 0.38 No report                         |
| (E)-9-Octadecenoic acid ethyl ester    | 21.83 | 310   | 1.04 No report                         |
| i-Propyl 11,12-methylene octadecanoate  | 22.23 | 338   | 0.16 Antimicrobial[68]                  |
| Ethyl Oleate                           | 22.57 | 310   | 0.36 Flavour[69].                       |
| Bis(2-ethylhexyl) phthalate            | 23.97 | 390   | 65.86 Antibacterial & antifungal[33]    |
| Docosanoic acid, ethyl ester           | 24.82 | 368   | 0.16 No report                         |
| Eicosanoic acid, 2-acetyloxy)-1-[acetyloxy)methyl]ethyl ster | 25.03 | 470   | 0.08 Antibacterial[70] antioxidans [71]|
| Eicosanoic acid, ethyl ester           | 25.61 | 340   | 0.56 No report                         |
| Cholestan-3-ol, 2-methylene-acetate, (3p,5a)- | 28.46 | 400   | 0.01 Antioxidant[72]                   |

**Conclusion:**

Aeromonas hydrophila have the potential of causing zoonotic disease, a disease spreads from animal to human being during accidental cases. Currently antibiotic treatment is preferred to resolve disease. But, use of antibiotics has
potential problem of being include inadequate dosage, overdosing, drug resistance by bacteria. Hence in the present study natural antimicrobial substance as a substitute for synthetic antibiotics is analyzed. Spirogyra extracts are prepared in different solvents with increasing order of polarity. Extracts are subjected to phytochemical tests for Glycosides,alkaloids, saponins,flavonoids,tannins,phenols,cardiac glycosides, sterols, resins etc. Extracts showing positive for phenol, tannins, flavonoids were estimated for phenolic content & tested for antimicrobial activity against pure cultures of Aeromonas hydrophila. Crude extracts subjected to GC –MS analysis reported many several bioactive compound showing antimicrobial, antioxidant & anti fungal property. The cold extraction procedure adopted helped in the accountability of lipidous& hydrocarbon molecule. Such natural antimicrobial substance showing broad spectrum activity can be used to replace synthetic antibiotics more effectively, less toxicity also can development of antibiotic resistant strains can be curtailed.

Acknowledgement:-
Author would like to thank Shri Dharmanathala Manjunatheshwara College(Autonomous) for providing laboratory ambience to carry out work.

References:-
1. V.L. Singleton and J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. Am. J. of Enol. Viticu. 6 (1965) 144-158.
2. Ely R, Supriya T, Naik CG. Antimicrobial activity of marineorganisms collected off the coast of South East India. J ExpMar Biol Ecol2004; 309: 121-127.
3. Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, TsayHS,Subbaraju GV. Assessment of bioactivity of Indian medicinalplants using brine shrimp (Artemiasalina) lethality assay. IntJAppSciEng2005; 2: 125-134.
4. Raghavendra MP, Satish S, RaveeshKA. Phytochemicalanalysis and antibacterial activity of Oxalis corniculata, a knownmedicinalplant. My Sci2006; 1(1): 72-78.
5. Selvamaleeswaran P, Wesely EG, Johnson M, VelusamyS,Jeyakumar N. The effect of leaves extracts of ClitoriaternateaLinnt against the fish pathogens. Asian Pac J Trop Med2010; 3(9):723-726.
6. Haripriya D, Selvan N, Jeyakumar N, Periasamy RS, Johnson M,rudayaraj V. The effect of extracts of Selaginellainvolvensand Selaginellinanequalifoliolaves on poultry pathogens. Asian Pac J Trop Med2010; 3(9): 678-681.
7. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituentsof some Nigerian medicinal plants. Afr J Biotecnol2005; 4:685-688.
8. Prashanthkumar P, Angadi SB, Vidyasagar GM. Antimicrobialactivity of blue green algae and green algae. Indian J Pharm Sci2006; 88(6): 647-648.
9. Singh AP, Chaudhary BK. Preliminary phytochemical analysissandin vitro anti-bacterial activity screening of Pithophoraoedogonia(Mont.) Wittrock-a fresh water green alga forming mats in the water bodies. J Algal Biomass Util2010; 1(2): 33-41.
10. Goud MJP, Seshikala D, CharyaMAS. Antibacterial activity andbiomolecular composition of certain fresh water microalgaefromRiver Godavari (India). Sci World J 2007; 2(3): 19-23.
11. Marhivanan K, Ramamurthy V, Rajaram R. Antimicrobial activityof Oscillatoria princes and Lyngbyja majuscule against pathogenicmicrorganisms. Int J Curr Res2010; 5: 97-101.
12. Parekh J, Chanda VS. In vitro antimicrobial activity andphytochemical analysis of some Indian medicinal plants. Turk JBiolog2007; 31: 53-58.
13. Transeau, E. N. (1951). The Zignematae. The Ohio State University Press, Columbus, Ohio, pp.327.
14. Kadłubowska, J. Z. (1972). Chlorophyta V. Conjugales: Zygmenaceae. Zrostnicowate. In: Starmach K, SieminskaJ(eds) Flora słodkowodnaPo Polski, PWN, Kraków, pp 431.
15. Goh S-H, Yusoff FM, Loh S-P (2010) A comparison of the antioxidant properties and total phenolic content in a diatom, Chaetoceros sp. and a green microalga, Nannochloropsis sp. J AgricSci2010; 123:130
16. Guzman S, Gato A, Galleja JM (2001) Antinflammatory, analgesic and free radical scavenging activities of the marine microalga Chlorella stigmatophoraphaeodactylum tricornutum. Phytother Res 15:224–230
17. Li H, Cheng K, Wong C, Fan K, Chen F, Jiang Y (2007) Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalga. Food Chem102:771–776
18. Miranda MS, Cintra RG, Barros SB, Mancini Filho J (1998) Antioxidant activity of the microalga Spirulina maxima. Braz J Med Biol Res 31:1075–1079
19. Mendiola J, Jaime L, Santoyo S, Reglero G, Cifuentes A, Ibanez E et al (2007) Screening of functional compounds in supercritical fluid extracts from Spirulinaplatensis. Food Chem 102:1357–1367
20. Cerón MC, García-Malea MC, Rivas J, Acien FG, Fernandez JM, Del Río E, Guerrero MG, Molina E (2007) Antioxidant activity of Haematococcus pluvialis cells grown in continuous culture as a function of their carotenoid and fatty acid content. ApplMicrobiolBiot 74:1112–1119
21. Rao AR, Sarada R, Baskaran V, Ravishankar GA (2006) Antioxidant activity of Botryococcus braunii extract elucidated in vitro models. J Agric Food Chem 54:4593–4599
22. Jaime L, Mendiola JA, Herrero M, Soler-Rivas C, Santoyo S, Señorans FJ, Cifuentes A, Ibáñez E (2005) Separation and characterization of antioxidants from Spirulina platensis microalga combining pressurized liquid extraction, TLC, and HPLC-DAD. J Sep Sci 28:2111–2119
23. Herrero M, Jaime L, Martín-Alvarez PJ, Cifuentes A, Ibáñez E (2006) Optimization of the extraction of antioxidants from Dunaliellasalina microalga by pressurized liquids. J Agric Food Chem 54:5597–5603
24. Singleton V.L., Rossi J.A., Colorimetry of total phenolics with phosphomolybdic – phosphotungstic acid reagents. Am. J. Enol. Vitic., 1965, 16, 144-158.
25. Parameswaran, P. S., Bhat, K. L., Das, B. N. &Kamat, S. Y. 1944. Halogenated terpenoids from the brown alga Padinatetrastramentica (Hauck). Indian Journal of chemistry. 33: 1006-1008.
26. Patterson, G. W. 1968. Sterols of Laminaria. Comparative Biochemistry and Physiology. 24:501-505.
27. Pratt, R., Daniel, T. C., Eier, J.B., Gunnison, J. B., Kumler, W. D., Oneto, J.F., Strait, L. A., Spoehr, H. A., Hardin, G. J., Milner, H. W., Smith, H. & Strain, H. H. 1944. Chlorellin. An antibacterial substance from chlorella. Science. 99:351-352.
28. González del Val A, Platas G, Basilio A (2001). Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). Int. Microbiol. 4:35-40.
29. [28] Snipes, W., Person, S., Keith, A. and Cupp, J. (1975) Science 188, 64-66. Brugh, M., jr (1977) Science 197, 1291-1292. Call, L.M., Sullivan, P.D., Nettleman, M.D., Ocasio, I.J., Blazyk, J. and Jollick, J. (1978) Biothem.Biophys. Res. Commun. 85, 351-356. Black, H.S., Chan, J.T. and Brown, G.E. (1978) Cancer Res. 38, 1384-1387. McCay, P.B., King,M.M. and Pitha, J.V. (1981) Cancer Res. 41, 3745-3748. Slaga, T.J. and Bracken, W.M. (1977) Cancer Res. 37, 1631-1635. P)
30. Antunes-Kenyon, Steven; Kennedy, Gerard.; Massachusetts. Pesticide Bureau. Methoprene: A review of the impacts of the insect growth regulator methoprene on non-target aquatic organisms in fish bearing water(Massachusetts Pesticide Bureau, Department of Food and Agriculture, 2001-08).
31. Agoramoorthy G, Chandrasekaran M, Venkatesaru V, Antimicrobial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India, Brazil J Microbiol, 2007; 38: 739-742.
32. R.N. Roy, S. Laskar, Dibutyl phthalate, the bioactive compound produced by Streptomyces albidosflavus 321.2 Microbiological Research 2006 Volume 161, Issue 2, Pages 121–126
33. Sermakkani M, Thangapandian V, gc-ms analysis of Cassiaitalica leaf methanolextract, Asian J Pharm Clin Res, 2012; 5(2):90-94
34. A. Kavitha, P. Prabhakar, M. Vijayalakshmi and Venkateswarlu Production of bioactive metabolites by Nocardialevis MK-VL 113 Article first published online: Jul 2009
35. Franck E. Dayan and Stephen O. Duke, Natural Compounds as Next Generation Herbicides, 2014, Plant Physiology Preview.
36. K. Ajaykumar., et al Journal of chemical & pharmaceuticals, 2015: Pyrans: Heterocycles of chemical & biological interest, 7(11): 693-700
37. Pavel C et al, Biological activities of Royal Jelly: Review: Animal sciences & Biotechnologies, 2011, 44(2)
38. Farina Mujeeb et al, Phytochemical evaluation, antimicrobial activity & determination of bioactive components from leaves of Aeglemarmelos, Biomed Research Institute, 2014
39. http://books.google.co.in http://www.jstage.jst.go.jp www.fisher.co.uk
40. BOJAR, R. A., HOLLAND, K. T. &CUNLIFFE, W. J. (1991). The in vitro antimicrobial effects of azelaic acid upon Propionibacterium acnes strain P37. Journal of Antimicrobial Chemotherapy 28, 843853.
41. www.hindawi.com/journals/bmri
42. Asma M. Alenad, Nabila A. Al-Jaber, Soundararajan Krishnaswamy, Sobhy M. Yakout, Nasser M. Al-Daghri and Majed S. Alokail. chileleaffragrantissima extract exerts its anticancer effect via induction of differentiation, cell cyclearrest and apoptosis in chronic myeloid leukemia(CML) cell line K562. Journal of Medicinal Plants Research, Vol. 7(21), pp. 1561-1567, 3 June, 2013
43. Raju K Chalannavar et al. Chemical constituent of essential oil from Syzygiumcordatum (Mystaceae). African Journal of Biotechnology. 2011. Vol.10(14) pg 2741-2745
45. Dinesh C Sharma et al. Phytochemical evaluation, antioxidant assay, antibacterial activity & determination of cell viability (J774 & THP1 alpha cell lines) of P. Sylvestris leaf crude & methanol purified fraction. EXCLI Journal 2016; 15:85-94
46. Vasudevan Aparna, Kalarickal V Dileep, Pradeep K Mandal et al., Anti inflammatory property of n-Hexadecanoic acid: Structural evidence & kinetic assessment. Chemical Biology & Drug design. Vol 80(3):434-439
47. Huang Chun Yan et al. Evaluation of antioxidant & antitumour activity of lemon essential oil. Journal of medicinal plant research. 2010, vol4(8): 1910-1915
48. Mustapha N. Abubakar and Runner R. T. Majinda. GC-MS analysis & preliminary antimicrobial activity of Albizia adiantifolia (Schumach) and pterocarpus angolensis (DC), Medicines 2016, 3, 3;
49. J/www.ncbi.nlm.nih.gov/pmc/articles/PMC3867356
50. www.academicjournals.org/article
51. www.springer.com/10.11862F513588
52. Nehal Singhal et al. Journal of chemical & pharmaceutical research ;2011, 3(2):126-133; Recent Advancement of Triazole derivatives & their biological significance.
53. Prem Janes et al., Antimicrobial activity of Diethyl pimelate an insilico approach; Journal of pharmaceutical clinical research 7(4)
54. Nisha Setal : Research &amp; Chemical research Journal 2015 ISSN 0975-8585; Phytochemicals screening &amp; GC MS analysis of Rhizomes of Maranta arundinacea L.
55. Vidhyamadhavi et al, Research journal of pharmaceutical biological sciences ISSN 0975-8585; Evaluation of invitro antioxidant, anti inflammatory properties of aerial parts of Zanthoxylum hirsuta
56. Microbiological Research vol170 :2015 pg 213-222; Identification & antifungal activity of novel organic compounds found in cuicatarial & internal lipids of medicinally important files
57. A U Isakhamyan et al: Pharmaceutical Chemistry Journal 47(9), 2013: Synthesis & some biological properties of amino alkyl ester methyloloolides of substituted acetic & propionic acids
58. Farzin H, IJBS 2007, 3(1):60-64. Synthesis & antitumour activity of substituted succinamides using a potato disc tumour induction assay
59. Sotifa A; 2012, 4(2); 1281-1287 chem composition & antimicrobial activity of essential oil & lipid content of Cardeus pyenocephalus L. growing in Saudi arabia
60. Sudha T et al. Journal of Applied pharmaceutical sciencesvol3(5)pg;126-130, 2013; GC MS analysis of bioactive components of aerial parts of Fluggea Leycoporus(wild)
61. Dan zhao et a, European journal of Medicinal chemistry vol 87, 2014 pg 500-507-Biological evaluation of halogenate thiazole[3,2-alpyrimidin-3-one, carboxylic acid derivatives argeting the YycGHistidine kinase
62. Meng-Fei Li et al electronic Journal of Biology, 2009, vol5(1)11-16; The dormancy mechanism & bioactivity of hydroquinone extracted from Podophyllum hexandrum royle seed
63. Rajeshwari R Nai; Oriental Journal of Chemistry. vol 31 ;GC –FID analysis of fatty acids 7 biological activity of Zanthoxylum hirsuta seed oil
64. Alazaablokatsya et al ; Silyl modification of biologically active compounds-13, synthesis, cytotoxicity & antibacterial action of N-methyl-N(2-triorg-arylisoxoxyethyl)-1,2,3,4-tetrahydro(iso)quinolinium iodies; Applied organometallic chemistry
65. Shodhganga.inflibnet.ac.in/chapter V;Synthesis Characterization & biological activity of novel pyrazoline derivative
66. G Rajeshwari et al Research journal of pharmaceutical biological & chemical sciences ISSN 0975-8585; GC-MS analysis of bioactive components of Hugonia mistax L.
67. Vigisaral ED et al, Journal of chemical & pharmaceutical Research 20146(8):294-300; GC-MS Analysis of bioactive constituents of Indigofera suffruticosa leaves
68. Denisa Liszekova, Maja Polakovicova, Milan Beno, Robert Farkas. Molecular Determinants of Juvenile Hormone Action as Revealed by 3D QSAR Analysis in Drosophila; PLoS:2009
69. MAH Nagalakshmi, K Sri Rama Murthy. Phytochemical Profile of Crude Seed Oil of Wrightiatinctoria R.BR. and Wrightiatarborea (DENNST.) MABB. by GC-MS Int. J. Pharm. Sci. Rev., 31(2), March 2015
70. Imad Hadi Hameed. Analysis of bioactive chemical compounds of Aspergillus niger by using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy Vol. 7(8), pp. 132-163, August 2015 DOI: 10.5897/JPP2015.0354 Article Number: 5F9D80B754311
71. Lawrence, B.M., Essential Oils 1976-1977, Essential Oils 1978, Essential Oils 1979-1980
72. Mishra Pratap Manjari, Sree Ayinampudi. Comparison of the antibacterial activity, volatiles and fatty acid composition of lipids of Phycopsis species collected at different locations from the Bay of Bengal (Orissa coast) Journal of the Serbian Chemical Society 2009 Volume 74, Issue 2, Pages: 133-139
73. Gopalakrishnan, Karikalan; RajangamUdayakumar. GC-MS Analysis of Phytocompounds of Leaf and Stem of Marsileaquadrifolia (L.) International Journal of Biochemistry Research & Review 4.6 (2014): 517-526
74. Faheem Amir*. Chemical Constituents and Biological Properties of the Marine Soft Coral Nephthea: A Review (Part 2) Tropical Journal of Pharmaceutical Research, June 2012; 11(3): 499-517
75. Austin B. & Austin D.A. (1989). Methods for microbial examination of fish and shellfish pp.317. Ellis Horwood Limited
76. LaDonswann & M Randy White DVM: Diagnosis & Treatment of *Aeromonashydrophila*’ Infection of fish’- Aquaculture extension. Illinos-Indiana Sea grant Programme