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Biochemical Profile and Inhibitory Effect of *Haliclona permollis* (Bowerbank, 1866) Marine Sponge of Ratnagiri, West Coast of India

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Abstract: *The intertidal marine sponge, Haliclona permollis was assessed for the antimicrobial effect of various crude extracts, against pathogenic microbes by agar well diffusion method as well as to determined preliminary biochemical screening. The methanol and acetone depicted strong positive antimicrobial activity. It may be due to the presence of alkaloids, glycosides, tannins, flavonoids, proteins and amino acids, steroids, carbohydrate, fats and fixed oil. The hexane and chloroform showed weak positive antimicrobial activity because presence of biologically active compounds in small quantity. The investigation indicated that Haliclona permollis remain an interesting source for antimicrobial activity and also suggest that could be a good source of the secondary metabolite. However it required further investigation for isolation of pure compound.*

Keywords: *Antimicrobial activity, Haliclona permollis, Biochemical profile, Intertidal, Pathogens.*

1. INTRODUCTION:

The marine sponges are the oldest metazoan group and characterized as sessile active filter feeders [1]. Sponges are simple, multicellular, sessile animals with no true tissue layers or organs [2]. This rocky shore area directly exposed to sea and it inhabited by diverse flora and fauna. Sponges are the most primitive multicellular animals that have existed for more than 800 million years. The sponges (Porifera), being evolutionarily ancient inhabit every type of marine benthic environment [3]. Sponges are primitive marine invertebrate's presence of high number natural products than any other marine phylum. The marine sponges are broadly distributed from intertidal zones to thousands of meters deep in the ocean [4].

The sponges are one of the richest sources of biologically active secondary metabolites and chemical diversity (5) (6). Until now, more than 5000 different compounds have been isolated and identified from about 500 species of sponges (7) with nearly 800 of them exhibiting antibiotic activity (8). These natural products belonged to different class of compounds like terpenoids, alkaloids, macrolides, polyether's, nucleoside derivatives and peptides. In recent time attention has been directed to the search of bioactive peptides from sponges, being actually a well-established sector in the research of marine natural product. Antitumor studies were conducted with 19 marine natural products in a number of experimental and clinical models proved that sponges act as an excellent source for bioactive compounds (9).

Marine sponges are a rich source of structurally novel and biologically active secondary metabolites [10]. Over 60% of potentially useful bioactive compounds discovered from living organisms have been obtained from marine fauna, 70% of which detected from sponges [11]. The sponge class Demospongiae is known for producing the largest number and diversity of secondary metabolites isolated from marine invertebrates [12]. Many sponge or sponge symbiont–derived metabolites are potent antibacterial, antifungal, anti-feeding and antifouling compounds [13]; a number of bacteria associated with sponges were found to be the sources of antibiotics and other bioactive compounds in the marine environment [14].

However, the bioactive potential of compounds from Indian sponges has been little studied, especially west coast of India. Therefore, In the present investigation report the antimicrobial and biochemical potential of marine intertidal sponge, *Haliclona permollis* collected from Ratnagiri coast (16°55'N73°16'E).

2. MATERIALS AND METHODS:

Collection of sample & preparation of crude extract-

The marine sponge, *Haliclona permollis* were collected from the low intertidal rocky pools of Ratnagiri coast (16°55'N 73°16'E), Maharashtra, India. The sponge was collected by an eco-friendly. Identified sponge tissues samples were washed with sea water, air dried and chopped into small size and extracted with 1000 ml (1:10) methanol, acetone, chloroform and hexane for about 7 days. Then extract was filtered through Whatmann paper No. 1 and solvent was processed by rotary vacuum evaporator (Buchi type-Superfit, Bangalore) under reduced pressure to get the crude extract of sponge. The concentrated extract was used for further study.

Antibacterial activity of *Haliclona permollis*

The assays were performed by agar well diffusion method is widely used to evaluate the antibacterial activity of crude extracts [15]. The four pathogenic bacterial strains were used as test organisms such as *Escherichia coli*, *Salmonella typhi*, (Gram negative bacteria) *Bacillus subtilis*, *Staphylococcus aureus* (Gram positive bacteria). All bacteria were stored at -20℃ until use. Cells were grown at 3^0C in Muller Hinton broth to an OD 420 = 1.9 (approx. 105 CFU/mL), and were transfer to Muller Hinton agar. The broth cultures swabbed onto agar medium so as to achieve a lawn of confluent bacterial growth separately for each strain. The sterile stainless steel borer (6 mm) was used to make well in the agar medium. Five wells were bored in each plate. The sponge crude extract (100µg /mL) was loaded in to the well and to find out the inhibitory potential. Triplicate plates were maintained for each test. Discs of Streptomycin (25µg/ml) were used as positive control. The bacterial assay plates were incubated at 37℃ for 24 hrs. Growth of bacteria around each well was observed carefully and the diameter of the zone of inhibition around each agar well was measured using a Hi-media zone reader.

Antifungal activity of *Haliclona permollis*

The assays were performed by agar well diffusion method is widely used to evaluate the antifungal activity of crude extracts [15]. Assays were performed by agar well diffusion method. The crude extract was tested against *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp. and *Fusarium* spp. The fungal cultures were maintained in 0.2% Sabouraud dextrose broth; each fungal inoculum was applied on plate and evenly spread on Sabouraud dextrose agar using a sterile cotton swab. The Fluconazole discs were used as the positive control. The sponge crude extract (100µg /mL) was loaded in to the well and to find out the inhibitory potential. The fungal assay plates were incubated at 28℃ for 48 hrs.

Preliminary biochemical screening of *Haliclona permollis*

The preliminary biochemical analysis was carried out using following methods [17, 18]. The sponge crude extracts were qualitatively analyzed for the presence of various biologically active compounds.

1. **Detection of alkaloids**

i. **Mayer's Test:** Extracts were treated with Mayer's reagent (potassium mercuric iodide). The formation of a yellow coloured precipitate indicates the presence of alkaloids in the extract.

ii. **Wagner's** Test: Extracts were treated with Wagner's reagent (Iodine in Potassium Iodide). The formation of a brown/reddish precipitate indicates the presence of alkaloids in the extract.

iii. **Dragendroff's Test:** Extracts were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). The formation of a red precipitate indicates the presence of alkaloids in the extract.

iv. **Hager's Test:** Extracts were treated with Hager's reagent (saturated picric acid solution). The formation of yellow coloured precipitate confirmed the Presence of alkaloids.

2. **Detection of glycosides**

Legal's Test: The extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. The pink to blood red colour indicates the presence of cardiac glycosides in the extract.

3. **Detection of tannins**

i. **Gelatin Test:** To the extract, 1% gelatin solution containing sodium chloride was added. The formation of a white precipitate indicates the presence of tannins in the extract.

ii. **Ferric Chloride Test:** With 1% ferric chloride solution the extract gives blue, green, or brownish green colour indicating the presence of tannins.

4. **Detection of flavonoids**

i. **Alkaline Reagent Test:** Extracts were treated with few drops of sodium hydroxide solution. The formation of intense yellow colour, it becomes colourless on addition of dilute acid indicates the presence of flavonoids in the extract.

ii. **Lead acetate Test:** Extracts were treated with few drops of lead acetate solution. The formation of a yellow coloured precipitate indicates the presence of flavonoids in the extract.

iii. **Shinoda Test:** Take 2-3 ml of extract, a piece of magnesium ribbon and 1 ml of conc. hydrochloric acid was added. The Pink or red coloration of the solution indicates the presence of flavonoids in the extract.

iv. **Zinc Hydrochloride Test:** To the test solution, add a mixture of zinc dust and conc. Hydrochloric acid. It gives red colour after few minutes.

5. **Detection of proteins and amino acids**

i. **Xanthoproteic Test:** The crude extracts were treated with few drops of concentrated nitric acid. The formation of a yellow colour indicates the presence of proteins.

ii. **Ninhydrin Test:** To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. The formation of a blue colour indicates the presence of amino acid.

6. **Detection of saponins**

Foam Test: Take the 0.5 gm of extract was shaken with 2 ml of water and Then formation of foam persistently for ten minutes it indicates the presence of saponins in the extract.

7. **Detection of sterols and terpenoids**

Salkowski's Test: Extracts were treated with few drops of concentrated sulphuric acid, red colour at the lower layer indicates presence of steroids and formation of yellow colour at the lower layer indicates the presence of terpenoids in the extract.

8. **Detection of carbohydrates**

i. **Molisch's** Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. The violet ring at the junction indicates the presence of Carbohydrates in the extract.

ii. **Benedict's Test:** Filtrates were treated with Benedict's reagent and heated gently. The orange red precipitate indicates the presence of reducing sugars in the extract.

iii. **Fehling's Test:** Filtrates were hydrolysed with diluted HCl, neutralized with alkali and heated with Fehling's A & B solutions. The formation of a red precipitate indicates the presence of reducing sugars in the extract.

iv. **Selwanoffs Test:** Take 1 ml of a sample solution of extract is placed in a test tube. The 2 ml of selwinoffs reagent (a solution of resorcinol and HCL) is added. The solution is heated in a boiling water bath for two minutes. The formation of red product indicates the presence of carbohydrates.

v. **Camnelisation Test:** 1 ml crude extract were treated with strong sulphuric acid, it gives a burning sugar smell. This indicates the presence of carbohydrates in the extract.

9. **Fats and Fixed Oils**

Stain Test: The small amount of extract was pressed between two filter papers. The oily stain on filter paper indicates the presence of fixed oil in the extract.

3. RESULTS:

The *Haliclona permollis* crude extracts methanol, acetone, chloroform and hexane were used to investigate the antimicrobial activity against four human pathogenic bacteria as well as four plant pathogenic fungal species; and the preliminary biochemical screening. Figure 1 shows result of in vitro testing of sponge extracts against pathogenic bacteria. Inhibition zones of sponge crude extracts against the specific test organisms were measured in mm. The crude extract restricted the growth of pathogens strains on the media around wells. The maximum inhibition zone (5-7 mm) was observed in methanol and acetone crude extract against *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Staphylococcus aureus*. The minimum inhibition zone (2-4 mm) was noticed in chloroform and hexane extract against all four pathogenic bacterial strains.

The figure 2 shows results of sponge crude extract against plant pathogenic fungal species. The maximum inhibition zone (5-7 mm) was observed in methanol crude extract against *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp. and *Fusarium* spp. and acetone extract shows (4-5) inhibition against *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp. and *Fusarium* spp.. The minimum inhibition zone (1-3.5 mm) was noticed in chloroform and hexane extract against all four pathogenic fungal strains.

The figure 3 to figure 10 depicted the various biochemical present in different extracts of sponge *Haliclona permollis;* the methanol and acetone crude extract contains alkaloids, tannins, flavonoids and proteins and amino acids, steroids, carbohydrates, fats and fixed oils strongly in high quantity; as well as chloroform and hexane extract contains presence of secondary metabolites in small quantity.

4. DISCUSSION:

In the present study the crude methanol, acetone, chloroform and hexane extracts of *Haliclona permollis* showed antimicrobial action against the bacteria and fungi. The crude extract of methanol shows maximum antimicrobial activity against all test microorganisms. The sponges shows wide spectrum of antibacterial efficacy and exhibited the growth of all the test bacteria. The reports on antibacterial activity of sponges revealed their activity on gram positive bacteria. Various studies have confirmed the predominance of gram negative producers in the marine environment [19]. Marine sponge *Aplysina cavernicola* produces the aeroplysinin, aerthionin derivatives, with some antibiotic activity against *Bacillus subtilis* and *Proteus vulgaris* [20].

Various studies have been done on anti–microbial properties of the bacteria associated with the sponges. The antibiotics produce by these bacteria ranged from broad spectral to species specific [21]. The discovery of new classes of antibiotics is necessary due to the increased incidence of multiple resistances among pathogenic microorganisms to

drugs that are currently in clinical use [22]. The Sponges of Demospongiae class are known to produce the largest number of secondary metabolitesss, most of them with medically relevant biological activities and important ecological roles [23].

Sponges are primitive marine invertebrate's present high number of natural products than any other marine phylum. Many of their products have strong bioactivities including anticancer, antimicrobial, larvicidal, hemolytic and anti-inflammatory activities and are often applicable for medical use [24]. The anti-tumour activity of cell free extracts from sponge associate actinomycetes might be due to the presence of the biologically active compounds alkaloids and guninine [25]. Hence, the present results profounded the promising antimicrobial activity of *Haliclona permollis* against eight active pathogenic strains. The study shows that *Haliclona permollis* possessed excellent source of antimicrobial properties and secondary metabolites.

5. CONCLUSION:

The present investigation reveals that the marine sponges *Haliclona permollis* shows the potential source for the antimicrobial and biochemical properties. The methanol and acetone depicted strong positive antimicrobial activity. It may be due to the presence of alkaloids, glycosides, tannins, flavonoids, proteins and amino acids, steroids, carbohydrate, fats and fixed oil. The hexane and chloroform showed weak positive antimicrobial activity because presence of biologically active compounds in small quantity. The investigation indicated that *Haliclona permollis* remain an interesting source for antimicrobial activity and also suggest that could be a good source of the secondary metabolite. Probably is the first report on the antimicrobial activity and biochemical profiling of *Haliclona permollis* from Ratnagiri coast, Maharashtra, India, to the best of our knowledge. However it required further investigation for isolation of pure compound.

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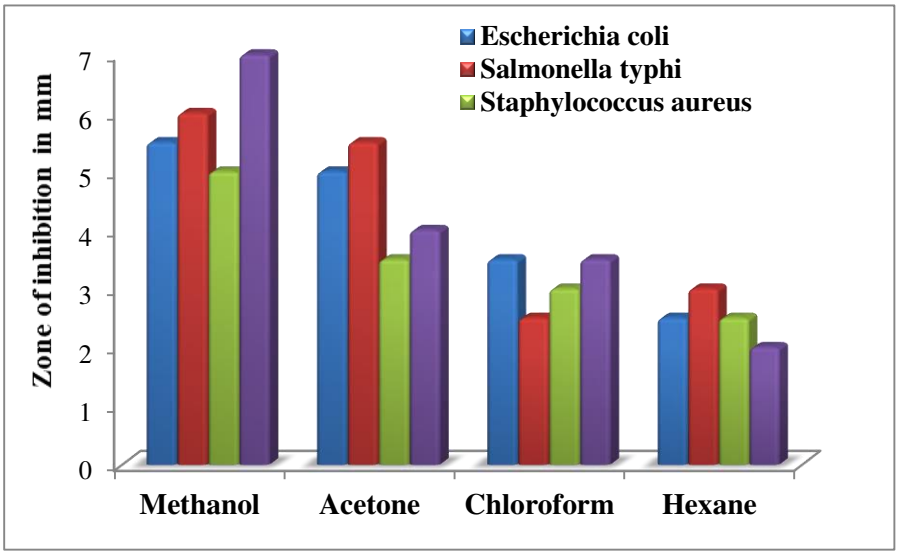

Figure 1: Antibacterial activity of crude extract of *Haliclona permollis.*

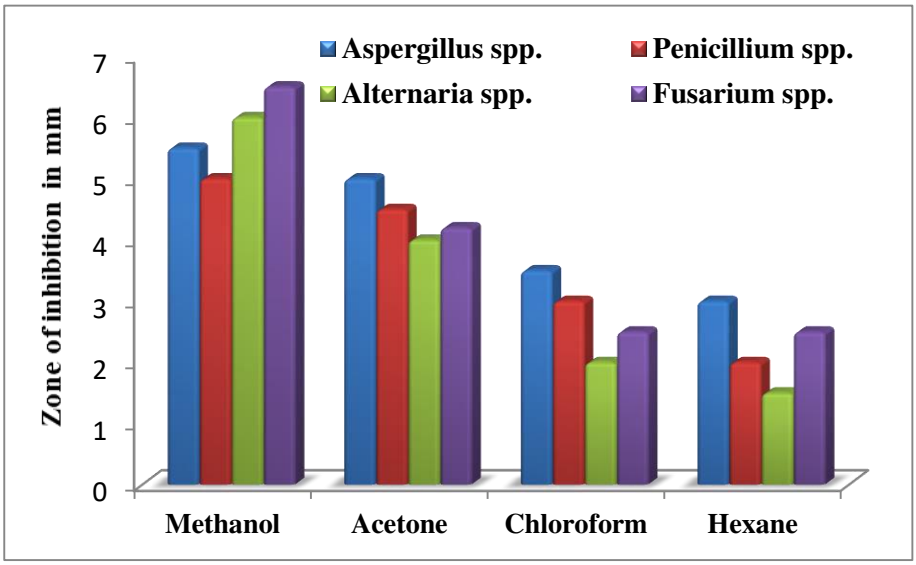

Figure 2: Antifungal activity of crude extract of *Haliclona permollis.*

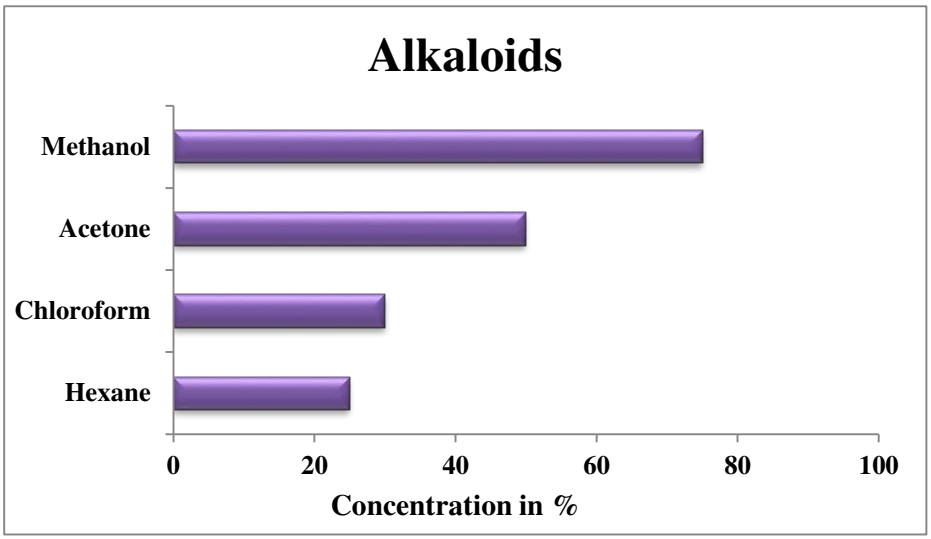

Figure 3: Alkaloid content in crude extracts of *Haliclona permollis.*

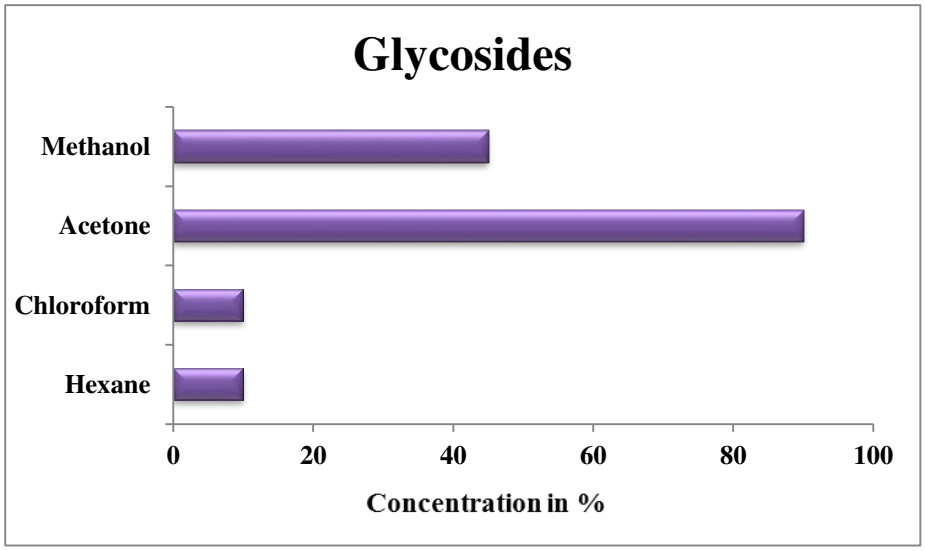

Figure 4: Glycoside content in crude extracts of *Haliclona permollis.*

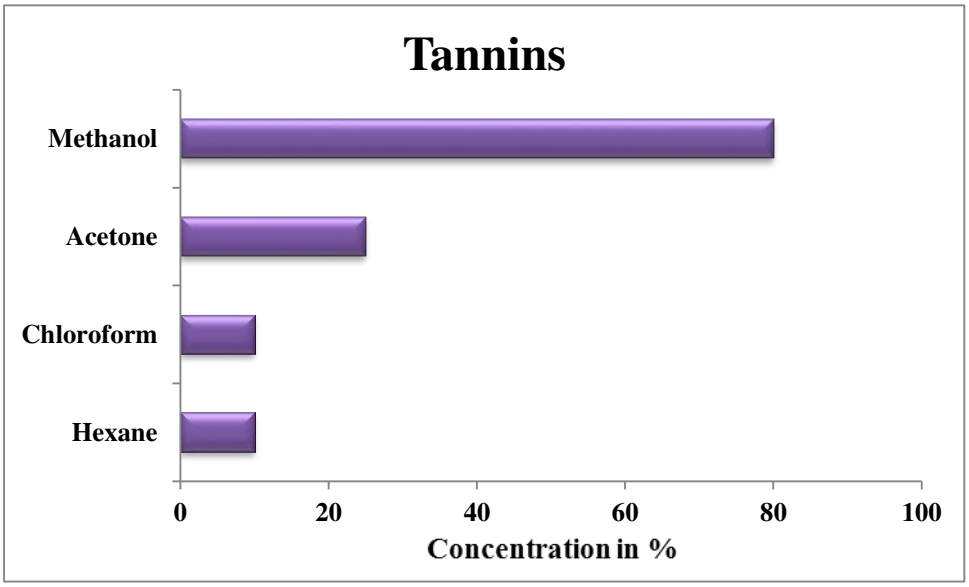

Figure 5: Tannin content in crude extracts of *Haliclona permollis.*

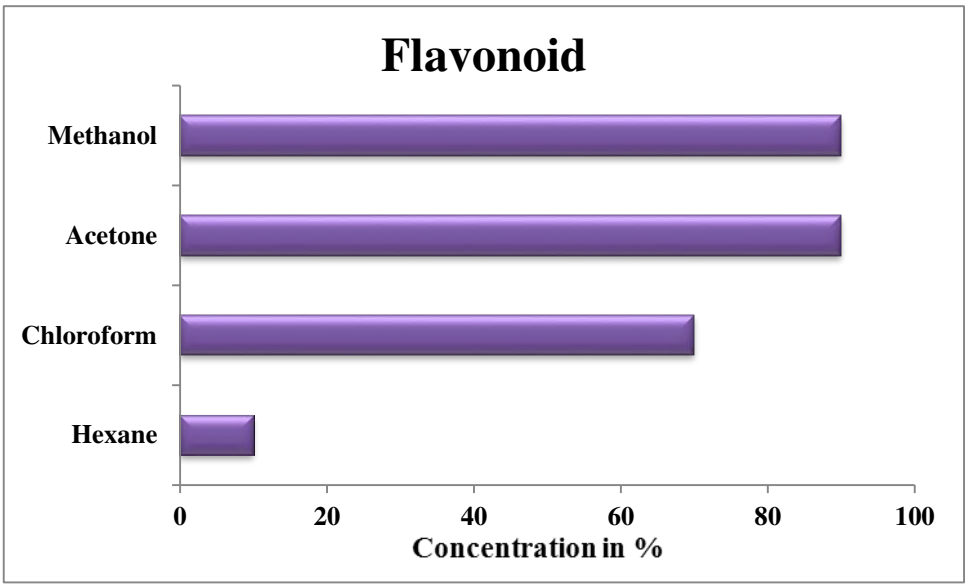

Figure 6: Flavonoid content in crude extracts of *Haliclona permollis.*

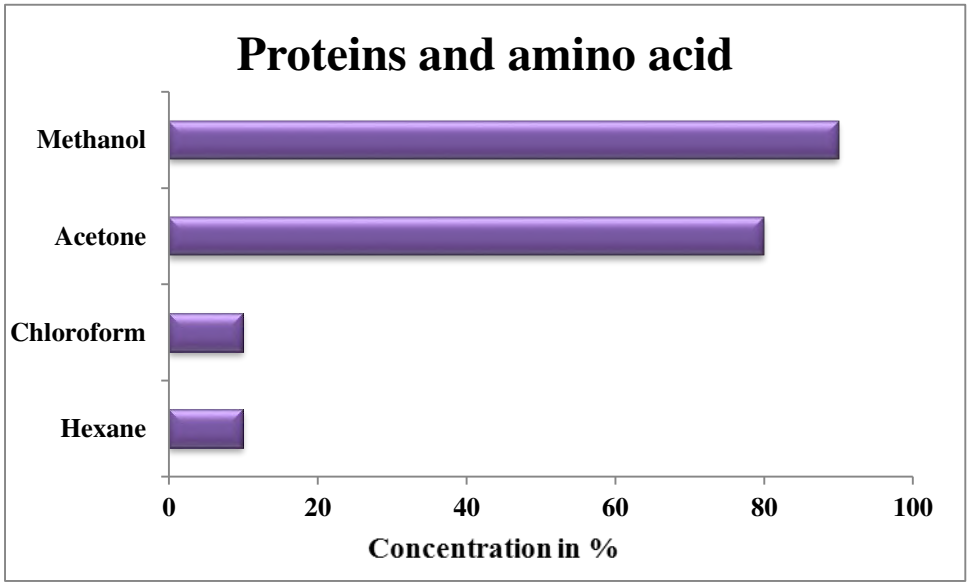

Figure 7: Proteins and amino acid content in crude extracts of *Haliclona permollis.*

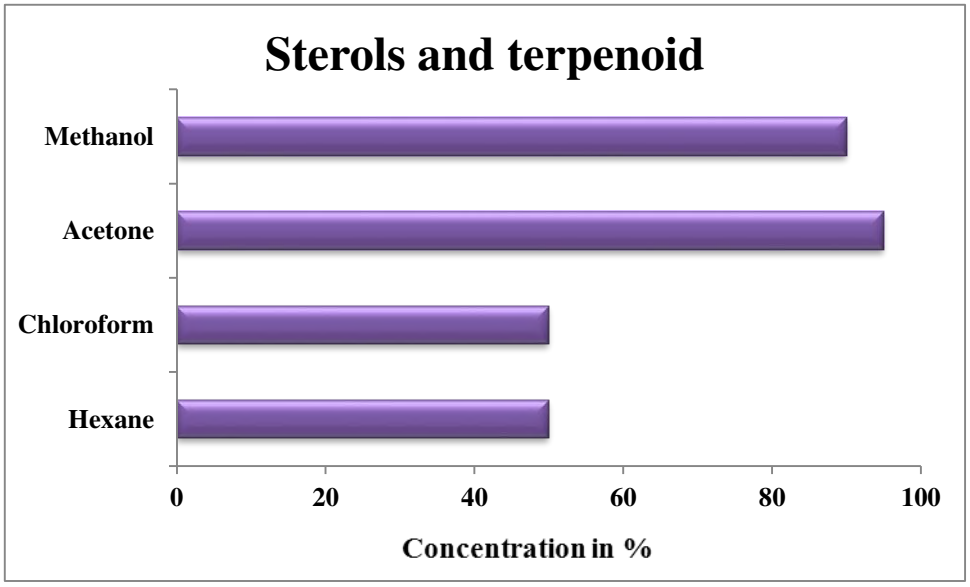

Figure 8: Sterols and terpenoid content in crude extracts of *Haliclona permollis.*

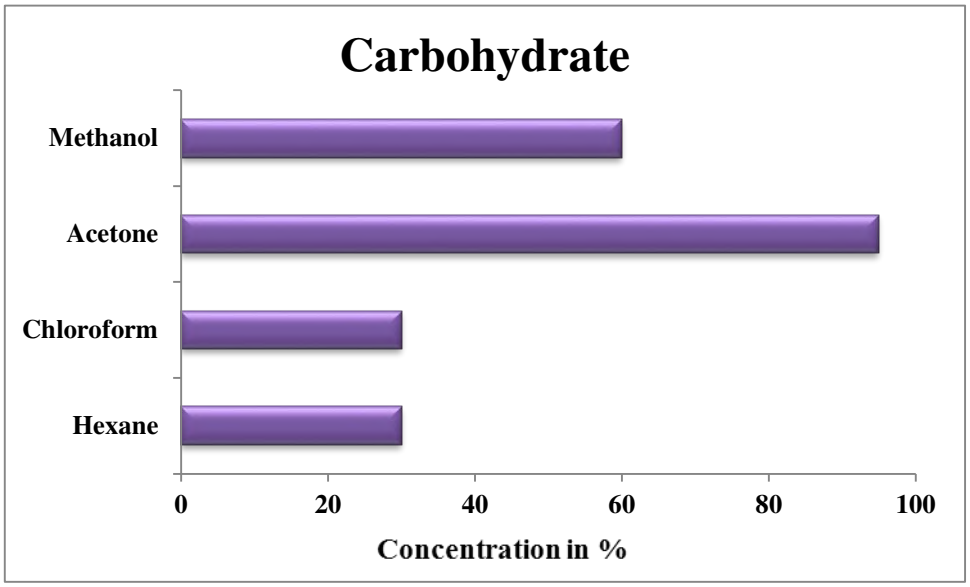

Figure 9: Carbohydrate content in crude extracts of *Haliclona permollis.*

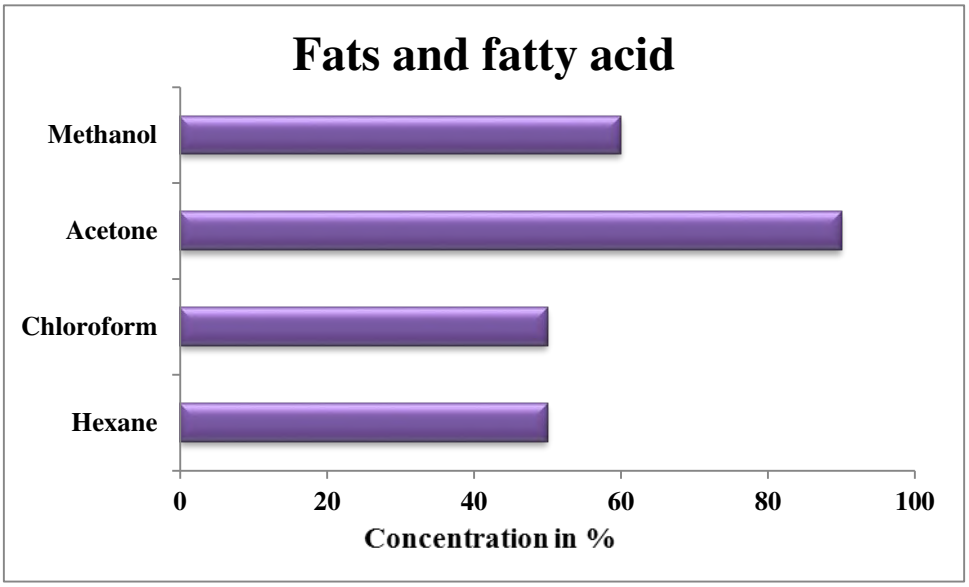

Figure 10: Fats and fatty acid content in crude extracts of *Haliclona permollis.*