# *In Vitro* Anti-leishmanial activity against cutaneous *Leishmania* parasites and preliminary phytochemical analysis of four Yemeni Medicinal Plants


## Abstract

Cutaneous leishmaniasis is one form of leishmaniasis that chiefly infected the poor sections of the society. The prototypical therapeutic interventions in vogue are handicapped due to toxicity and an alarming increase in drug resistance. Furthermore, the absence of vaccines has raised the quest for alternative therapies. So, the aim of our study was to assess the anti-leishmanial activity of *Euphorbia cactus* Ehrenb, *Euphorbia ammak* Forssk, *Euphorbia inarticulate* Schweinf, and *Pergularia tomentosa* L. The extracts of plants were prepared by maceration method and by Soxhlet extractor. The extracts were dried and re-dissolved in 2% dimethyl sulfoxide (DMSO) 1% solvent. *Leishmania spp*. cells were then tested with serial concentrations (15.6 $\mu gml^{1}$ to 500 $\mu gml^{1}$ ) of the extracts, using the 3-(4,5-dimethylthazolk-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. All experiments were performed in triplicate and analyzed by ANOVA test. The optical density values as measured by Enzyme-Linked Immunosorbent Assay (ELISA) were used to calculate the $IC_{50}$ values. The results indicated that the methanolic latex extract of *Euphorbia cactus* Ehrenb, *Euphorbia ammak* Forssk had potent anti-leishmanial activity against the promastigotes of *Leishmania* spp. based on a dose-dependent response analysis. The $IC_{50}$ values for *Euphorbia cactus* Ehrenb and *Euphorbia ammak* Forssk after 24h incubation against *Leishmania* spp. promastigotes were less than <15.6 $\mu gml^{-1}$. Furthermore, the phytochemical analysis of methanolic extract showed the presence of alkaloids, phytosterols, phenols, saponins, and flavonoids in which these components have been proven previously to be the active compounds against *Leishmania* parasite. In conclusion, the present study reveals that latex extract of *Euphorbia cactus* Ehrenb and *Euphorbia ammak* Forssk contain active compounds that have anti-leishmanial activity, which could serve as an alternative agent in the treatment of cutaneous leishmaniasis, but further studies would, therefore, be needed to assess the activity of these materials of this plants *in vivo* clinical response and study their toxicity on cell lines.


**Keywords:** anti-leishmanial activity, *Euphorbia cactus* Ehrenb, *Euphorbia ammak* Forssk, *Euphorbia inarticulate* Schweinf, *Pergularia tomentosa* L, Yemeni Medicinal Plants.



## Introduction

Cutaneous leishmaniasis (CL), one clinical form of leishmaniasis, a term referred to skin clinical symptoms caused by several species of obligate intra-monocular phagocytic cells protozoan parasites produce a skin ulcer that heals spontaneously in most cases leaving an unsightly scar . These parasites belong to the genus *Leishmania*, that transmitted by the bite of a female phlebotomine sand fly.[1-4] Cutaneous leishmaniasis is still one of the world's most neglected disease that significant morbidity worldwide and shows a worrying increasing trend. CL is endemic in large areas of the tropics, subtropics, and the Mediterranean basin.[5] In Yemen**,** this disease is endemic and the most prevalent skin infectious diseases.[6,7] To date progression in developing an effective vaccine against CL has not been successes

and chemotherapy is the only effective way to treat the disease. However, current therapy is toxic, expensive, have severe side effects, as well as it emerges a resistance to drugs. Therefore, there is a great and urgent need for developing a new and safe anti-leishmanial drug.[8-11]

Investigation bioactive compounds from plants that used medicinally are regarded as one of the strategies to discover new drugs for leishmaniasis.[12] The World Health Organization (WHO) has estimated that approximately 80% of individuals rely on traditional medicines for their primary health care needs.[13,14] In different cultures and countries, many plants are used in the form of powders, crude extracts or infusion to treat several diseases including parasitic diseases without any scientific evidence of efficacy. In Yemen, there is a rich tradition of the use of herbal medicine for the treatment of various diseases, including inflammations, infections and other diseases.[15-18]

Despite the worldwide spread of Cutaneous leishmaniasis and the significant morbidity that caused by this disease in the world as well as in Yemen, its current drugs have limitations. So the investigation of plants that are used in folk medicine may have prognostic value to discover new and safe cutaneous leishmanicidal drug. The present study conducted to offer a scientific basis for the traditional use of *Euphorbia cactus* Ehrenb, *Euphorbia ammak* Forssk, *Euphorbia inarticulate* Schweinf, stems *and* leaves of *Pergularia tomentosa L* as cutaneous leishmanicidal, this may be regarded as future promising phytotherapeutics in the treatment cutaneous leishmaniasis. The objectives of this study were to evaluate the *in vitro* cutaneous anti-leishmanial activity of *Euphorbia cactus Ehrenb, Euphorbia ammak* Forssk, *Euphorbia inarticulate* Schweinf, *stems and leaves of Pergularia tomentosa L* extracts.Also carrying out preliminary phytochemical screening of those plant extracts.



**Subjects and Methods**

**Plant materials**

Four selected plants (*Euphorbia cactus* Ehrenb, *Euphorbia ammak* Forssk, *Euphorbia inarticulate* Schweinf, stem and leaf of *Pergularia tomentosa* L *(*table 1) commonly used in Yemen by traditional healers for the treatment of cutaneous leishmaniasis and other skin diseases were collected from Taiz governorate of Yemen in September 2016, and botanical identification was by Dr. Hassan Ibrahim botanist at the Botany section, Biology department, Faculty of Science, Sana'a university, Sana'a (Yemen).

**Plant preparation**

Stems and leaves of *Pergularia tomentosa* L and phylloclade of *Euphorbia inarticulate* Schweinf were collected, washed, sliced, weighed and sundried under the shade at room temperature. After complete drying , they grinded to a coarse powder in electrical blender. The dried crude plants were maintained in dark vials and stored at -20 °C until used.[19,20] The latex of *Euphorbia cactus* Ehrenb and *Euphorbia ammak* Forssk were collected in clean glass bottles and were kept in the refrigerator (4°C – 8°C) until extraction.[20]

**Plants extraction:**

The dried plants were extracted successively with MeOH (80%) by using a Soxhlet apparatus**.** The obtained extracts were filtered through a Whatman-1 filter paper. The filtrates were dried by evaporation on a rotary evaporator below 45˚C and freeze dryer to give the crude dried extract. All extracts were stored at -20 $^{o}$C until used, the yield obtained are shown in Table 2.

Latex were extracted by maceration using MeOH(80%). The latex were soaked (1 ml) in 10 ml of solvent in a stoppered container with frequent agitation at room

temperature. After maceration the soaked latex were filtrated through a Whatman-1 filter paper. The filtration were dried by a rotary evaporation and freeze drier to give the crude dried extract. The crude dried extract of latex were stored at – 20, the yield obtained are shown in Table 2.

**Anti-promastigote assay**

**Patient selection and *Leishmania* spp. isolation:**

Following clinical diagnosis by dermatologist, and confirmed by laboratory. *Leishmania* spp. were isolated from the patient with cutaneous leishmaniasis infection. Skin lesions of the patients were cleansed with 70% ethanol before sample scraping. These preparations were stained with Giemsa and examined under a light microscope with magnification (×1000), and it was inoculated in Nicolle-Novy-McNeal (NNN) culture medium. The culture tubes were kept in an incubator at $25^0$C for 5-10 days. *Leishmania* spp. promastigote that observed during microscopy transferred to RPMI-1640 and incubated at 25 °C for mass cultivation medium. Patient who included in this study were : (1) patient with infection not exceed 6 months, (2) patient did not used drugs or herbs, (3) Heavy infection .

**Determination of 50% promastigote growth inhibitory concentration ($IC_{50}$) of the plant extracts**

Stock solution of crude extracts was prepared in 2 % (DMSO)/Deionized water at 10 mg/10mL. RPMI-1640 medium were distributed in each well of a 96 well plate. The extract solutions (100µl) were serially diluted down each lane of 96-well plate with medium. Then, the growing cells at $10^6$ promastigotes/ml were added to each well to give final six concentration of plant extracts 15.6µgml$^{-1}$, 31.25 µgml$^{-1}$, 62.56 µgml$^{-1}$, 125 µgml$^{-1}$, 250 µgml$^{-1}$, 500 µgml$^{-1}$ (two folds dilutions). The final concentration of DMSO was not be higher than 1% (v/v) as this concentration did not affect the parasite growth rate, mobility, morphology[21] in all experiments. A Pentostam (Albert David Limited-India) was used as positive control at 10µgml$^{-1}$, untreated media were used as negative control, and DMSO alone was used as solvent control. 96-well micro-plates were incubated at $26^0$C. After the incubation, the effect of the isolated extracts on cell viability was assessed using the 3-(4,5-dimethylthazolk-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, which measures the metabolic activity of mitochondria.[22] In which, 20µL of MTT solution (5mgmL$^{-1}$) (SIGMA) was added to each well and incubated at 26◦C for 4 h at 26 ˚C. The test principle "Tetrazolium salts are cleaved to formazan dye by cellular enzyme mitochondrial succinate dehydrogenase (only in the viable promastigites)" . Finally, the MTT solubilization solution was added to each well to dissolve the insoluble purple formazan product into coloration solution and incubated for another 10 min. The absorbance was measured at 570 nm for each well, using an ELISA reader.[23,24] The live promastigotes percentage inhibition ratio were calculated as described by Bansal *et al*..[25]

**Inhibition Rate (I%):**

Data were presented as mean±SD of triplicate experiments for each well. Mediam inhibitory concentration value ($IC_{50\ value}$), the concentration that decreased parasite growth by 50% was calculated using linear regression analysis (dose – response analysis) associated with 95% confidence interval. Lower IC50 value indicates greater anti-leishmanial activity.

***Leishmania* spp. counting**

20 µl of cells from the culture were taken and placed in 1.5 ml Eppendorf tube containing 20 µl of Eosin (1%) then mixed. After that 10µl of cells were placed on

haemocytometer (Thoma slide). 5 squares were counted then the result number were multiply by 100000 so we can get the number of cells in 1 ml of culture .

**Preliminary Qualitative Phytochemical Screening**

The dried extracts were tested to identify alkaloids, carbohydrates, glycosides, fixed oil and fats, anthraquinones, phenolic compounds and tannins, phytosterols, proteins, saponins, gum and mucilage. A preliminary phytochemical analysis was carried out using tube-test reaction and thin-layer chromatography (TLC) as described by Banu and Cathrine.[19]

**Statistical Analysis**

Data are presented as mean ± SD of triplicate experiments , and the plant extract concentration required for 50% inhibition *in vitro* ($IC_{50}$) calculated by dose-response analysis associated with 95% confidence interval by Graph pad prism 7 Demo.

**Ethical Consideration**

Ethical clearance for the study was taken from the Faculty of Medicine and Health Sciences Research Review Committee. Informed Consent was taken from the patients before the collecting specimens.

**RESULTS**

The percentages of extraction yield for each investigated plant were summarized in Table 2. The results of anti-Promastigote assay (in vitro test) for the five plant extracts against promastigotes stages of *Leishmania* spp. are summarized in Table 3,4, 5, 6,7. Data in Table 8 elucidate that the highest antileishmanial activity was obtained from *Euphorbia cactus* Ehrenb and *Euphorbia ammak* Forssk with $IC_{50}$ value < 15.6 $\mu gml^{-1.}$ The leaves and stems of *Pergularia tomentosa* L and *Euphorbia innerticulata* Schweinf extracts showed inactive antileishmanial activity ($IC_{50}$ value > 500 $\mu gml^{-1}$).

**Phytochemical analysis and thin layer chromatography results**

Phytochemical analysis of extracts indicated the presence of various bioactive components. Compounds, such as alkaloids were found in the five plant extracts. However, fixed oils, fats and Anthraquinones were absent in all extracts, Phytosterols were found in the four plant extract and in *Euphorbia ammak* Forssk extract was absent. Glycosides, Gum and Mucilage were found only *in Euphorbia cactus* Ehrenb and *Euphorbia ammak* Forssk, Protins were found only in *Euphorbia cactus* Ehrenb and *Euphorbia inarticulate* Schweinf. However, Saponines were found only in *Euphorbia cactus* Ehernb, *Euphorbia ammak* Forssk, stem and leaf of *Pergularia tomentosa L*, and carbohydrates were found in *Euphorbia inerticulata* schweinf, stem and leaf of *Pregularia tomentosa* L (Table 9) . The TLC analysis showed spots of determined constitutes in each extract (Table 10).

**Discussion**

The first choice drug for treatment of cutaneous leshmaniasis is Pentostam which has toxic side effects. Also, no vaccine is available to cure this disease. Most of the studies directed towards plants that is used traditionally as potential source of new alternative medicines. Some of the drugs obtained from plants used in the treatment of diseases caused by protozoan include alkaloids quinine obtained from the plant genus *Cinchora* and artemisinin obtained from the plant genus *Artemisia annua* and both of them used in the treatment of malaria. As well as, emetin obtained from the plant genus *Cephaelis* used in treatment of ameobiasis.[26] Our findings showed that the methanol latex extract of *Euophobia cactus Ehrenb* (Euophrbiaceae) had choice antileshmanial activity (IC50<15.6 $\mu gml^{-1}$ against promastigotes). There was no

previous studies reported about its biological activities against *Leishmania* spp. to compare with, so our results appear to be one of the first that studied its activity. The methanol latex extract of *Euphorbia ammak Forssk* showed a good antileishmanial activity (IC50<15.6µgml$^{-1}$ against promastigotes). Our data is compatible with data reported by Abdel-Sattar *et al.* (2010) against *Leishmania infantum* which found to inhibit the growth of *Leishmania infantum* with IC50 value < 24.05 µgml$^{-1}$.[27] These findings confirmed that latex of *Euophobia cactus* Ehrenb and *Euphorbia ammak* Forssk could be considered as having promising antileishmanial activity.

The methanol extract of *Euphorbia inarticulate* Schweinf phylloclades showed no antileishmanial activity (IC50>500 µgml$^{-1}$ ). Although, there was no previous studies reported about its biological activities against *Leishmania* spp. to compare with, so our results appear to be one of the first that study its antileishmanial activity.

The leaves and stems extracts of *Pergularia tomentosa L* and *Euphorbia inarticulate* Schweinf showed no antileshmanial activity (IC50>500 µgml$^{-1}$ ), while several studies reported good activity of these plants against fungi.[28,29]

Flavnoids may be the active compound in *Euphorbia ammak* Forssk against *Leishmania* spp. in our study. Our findings can be confirmed by the finding of Das *et al.*[30] in which they tested the antileishmanial activity of flavonoids against topoisomerase I of *Leishmania donovani*. They illustrated that these compounds inhibited topoisomerase I which subsequently inhibit the relegation step in parasite growth.[30]

Phenolic, saponins, and alkaloids that we detected in our study may be they are the active compounds responsible for antileishmanial activity in *Euphorbia ammak* Forssk and *Eiphorbia cactus* Ehrenb. Kayser *et al.*[31] in their comprehensive review of antiparasitic drug development view phenolic, saponins, and alkaloids have inhibition activity against *Leishmania* parasite.[31, 32]

Phytochemical results of leaves and stems of *Pergularia tomentosa* L indicated the presence of saponins, phytosterols, phenolic compounds, tannins, carbohydrates, alkaloids, and flavonoids, except coumarins only present in stems. The phytochemical results of leaves and stems of *Pergularia tomentosa* L in our study is in agreement with Hassan *et al.* (2007) and Shinkafi (2014) in Nigeria, except glycosides, and anthraquainins were not present in our extracts of *Pergularia tomentosa L.*[28, 29] The absent of glycosides, and anthraquainins in our plant *Pergularia tomentosa L* might be due to the environmental factors, such as altitude, temperature, illumination, precipitation, humidity, soils and locations as described by Zidorn and Stuppner (2001) in which they referred these differences in components is due to the environmental factors.[33]

**Conclusion**

In conclusion, the methanolic extract of *Euphorbia cactus* Ehrenb *and Euphorbia ammak* Forssk showed a good *antileishmanial activity with IC50 value>15.6 µgml$^{-1}$* relative to negative control. The preliminary phytochemical investigation reveal the extracts contain secondary metabolites, that indicate these plants may be highly promising candidate drugs. Furthermore the results offer a scientific basis for the traditional use of investigated plants. This is a preliminary evaluation using promastigotes must be complemented with an evaluation using intracellular amastigotes in macrophages. At the same time, an evaluation of the possible cytotoxicity of the tested plants is important. Further screening for Yemeni plants species, especially that are used in traditional medicine must be done for searching of potential anti-leishmanial active constituents and record ethno-botanical data.

**Acknowledgments:**

The authors would like to acknowledge Tropical Disease Research Center at Technology & information university, Faculty of Pharmacy, Sana'a University , and the Microbiology Department of the National Center of Public Health Laboratories (NCPHL) and Yemen Lab, Sana'a, Yemen which provided working space. Thanks go to Musned Education fund for financial supporting.

**Conflict of interest:**

"No conflict of interest associated with this work".

**Author's contribution**

This research work is part of A M.Sc. thesis. The candidate is the first author(MMA) who conducted experiments and wrote up the thesis. The corresponding author (HAA) supervised the experimental work, revised and edited the thesis draft and the manuscript. (BYA) was co-advisor of the work, and (BAM) helped in chemical analysis.

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**Table 1: List of the selected plants that used in the investigation**

| Plant species (family) | Local name | Plant part used [a] | Site of collection |
|---|---|---|---|
| *Euphorbia cactus* Ehrenb (Euphorbiaceae) | اكرث, كرثي, كرث, صال , عشبق, قلح, عمق | **Lat.** | North Taiz |
| *Euphorbia ammak* Forssk (Euphorbiaceae) | عمقه, عمق | **Lat.** | North Taiz |
| *Euphorbia inarticulate Schweinf* (Euphorbiaceae) | قصي, اقصص, قصاص, صيب, كلح, محاط, خريش | **Pha.** | North Taiz |
| *Pergularia tomentosa L* (Asclepiadeceae) | غلقه, غلق, كابيش, دامية | S,L | North Taiz |

a- S. :Stem, Pha. : phylloclades, L:Leaves , Lat.: Latex.

**Table 2: Percentage extraction yield of plant extracts.**

| No. | Plant name | Crude methanolic extract (g) | Amount of plant extracted(g) and yield of extraction(%) |
|---|---|---|---|
| 1 | *Euphorbia cactus* Ehrenb | 5.24 | 206 (2.54%) |
| 2 | *Euphorbia ammak* Forssk | 11.215 | 309 (3.629%) |
| 3 | *Euphorbia inarticulate* Schweinf | 32.870 | 350 (9.39%) |
| 4 | *Pergularia tomentosa L* leaves | 29 | 300 (9%) |
| 5 | *Pergularia tomentosa L* Stems | 43.98 | 300 (14.66%) |

**Unit of latex express as g.**

**Table 3: *In vitro* l activity of *Euphorbia cactus* Ehrenb methanolic extracts on *Leishmania* spp. after 24h**

| Concentration | Parasite inhibition mean±SD* | Inhibition% |
|---|---|---|
| 15.6µg/ml | **0.034±0.045[a,b]** | **80.5** |
| 31.25µg/ml | **0.034±0.046[a,b]** | **80.5** |
| 62.56µg/ml | **0.029±0.037[a,b]** | **84.32** |
| 125µg/ml | **0.004±0.018[a,b]** | **92.37** |
| 250µg/ml | **0.007±0.0176[a,b]** | **92.54** |
| 500µg/ml | **0.004±0.017[a,b]** | **92.79** |
| Untreated control | 0.008±0.236 | |

Mean ±SD is OD values of 3 wells. * Average ± standard division, [a] No significant difference compared to each other (P>0.05). [b] Significant difference compared to untreated wells (P<0.05)

**Table 4: In vitro activity of *Euphorbia ammak* Forssk methanolic extracts against *Leishmania* spp. after 24h.**

| Concentration | Parasite inhibition (mean±SD*) | Inhibition% |
|---|---|---|
| 15.6µg/ml | 0.088±0.006[b] | 62.71 |
| 31.25µg/ml | 0.089±0.004[b] | 62.28 |
| 62.5µg/ml | 0.084±0.002[b] | 64.4 |
| 125µg/ml | 0.075±0.001[b,c] | 68.22 |
| 250µg/ml | 0.067±0.007[b,c] | 71.61 |
| 5006µg/ml | 0.068±0.001[b,c] | 71.18 |
| Untreated control | 0.008±0.236 | |

Mean ±SD is OD values of 3 wells. * Average ± standard division, [b] Significant difference compared to untreated wells (P<0.05). [c] significant difference compared to 15.6, 31.25, and 62.5µg/ml (P<0.05).

**Table 5*: In vitro* activity of *Euphorbia inerticulata* Shweinf extracts against *Leishmania* spp. for 24h**

| Concentration | Parasite inhibition (mean±SD*) | Inhibition% |
|---|---|---|
| 15.6µg/ml | 0.164±0.033[b] | 30.5 |
| 31.25µg/ml | 0.155±0.016[b] | 34.32 |
| 62.56µg/ml | 0.154±0.015[b] | 34.74 |
| 125µg/ml | 0.142±0.012[b] | 39.83 |
| 250µg/ml | 0.141±0.01[b] | 40.25 |
| 500µg/ml | 0.119±0.008[b,c] | 49.59 |

Mean ±SD is OD values of 3 wells. * Average ± standard division, [b] Significant difference compared to untreated wells (P<0.05). [c] significant difference compared to 15.6µg/ml (P<0.05).

**Table 6*: In vitro* activity of *Pergularia tomentosa L.* leaves methanolic extracts against *Leishmania* spp. for 24h.**

| Concentration | Parasite inhibition (mean±SD*) | Inhibition% |
|---|---|---|
| 15.6µg/ml | **0.172±0.03[a,b]** | **27.11** |
| 31.25µg/ml | **0.168±0.022[a,b]** | **28.81** |
| 62.56µg/ml | **0.155±0.021[a,b]** | **34.32** |
| 125µg/ml | **0.151±0.001[a,b]** | **36.01** |
| 250µg/ml | **0.146±0.021[a,b]** | **38.13** |
| 500µg/ml | **0.146±0.033[a,b]** | **38.13** |
| Untreated control | 0.008±0.236 | |

Mean ±SD is OD values of 3 wells. * Average ± standard division, [a] No significant difference compared to each other (P>0.05). the [b] Significant difference compared to untreated wells (P<0.05)

**Table 7: *In vitro* activity of *Pergularia tomentosa L. stems* methanolic extracts against *Leishmania* spp. for 24h**

| Concentration | Parasite inhibition )mean±SD(* | Inhibition% |
|---|---|---|
| 15.6µg/ml | **0.001±0.207** | **12.28** |
| 31.25µg/ml | **0.001±0.203** | **13.98** |
| 62.5µg/ml | **0.008±0.189**[b,c] | **19.91** |
| 125µg/ml | **0.004±0.191**[b,c] | **19.06** |
| 250µg/ml | **0.004±0.171**[b,c] | **27.54** |
| 5006µg/ml | **0.012±0.151**[b,c] | **36.01** |
| Untreated control | **0.008±0.236** | |

Mean ±SD is OD values of 3 wells. * Average ± standard division, [b] Significant difference compared to untreated wells (P<0.05). c significant difference compared to 15.6 and 31.25 µg/ml (P<0.05).

**Table 8: $IC_{50}$ values(µg/ml) for promastigotes growth inhibition of the methanol extracts of five plant.**

| **Plant extracts** | **IC50µg/ml** |
|---|---|
| *Euphorbia cactus* Ehrenb | <15.6 |
| *Euphorbia ammak* | <15.6 |
| *Euphorbia innerticulata* Schweinf | >500 |
| *Pergularia tomentosa L.* L | >500 |
| *Pergularia tomentosa L.* S | >500 |

$IC_{50}$=Inhibitory concentration 50%, L= leaves, S= stems.

**Table 9: Qualitative phytochemical analysis of plant extracts by chemical method.**

| **Constituents** | **Test** | **E.C** | **E.A** | **E.I** | **P.L** | **P.S** |
|---|---|---|---|---|---|---|
| Alkaloids | Mayer's test | + | + | + | + | + |
| | Wanger's test | + | + | + | + | + |
| Carbohydrates | Bendict's test | - | - | + | + | + |
| Fixed oil & fats | Spot test | - | - | - | - | - |
| Glycosides | Salkowski's test | + | + | - | - | - |
| Anthraquinones | Borntrager's test | - | - | - | - | - |
| Phenolic compounds & Tanins | Ferric chloride test | - | - | + | + | + |
| | Mg and HCL reduction test | - | - | + | + | + |
| Phytosterols | Libermann-Burchard's test | + | - | + | + | + |
| Proteins | Biuret test | + | - | + | - | - |
| Saponins | Foam test | + | + | - | + | - |
| Gum andMucilage | Gum and Mucilage test | + | + | - | - | - |

+= signify presence , -= signify Absence, E.C= *Euphorbia cactus*, E.I= *Euphorbia inerticulata*, E.A= *Euphorbia ammak*, P.L= *Pergularia tomentoa L* leaves, P.S= *Pergularia tomentoa L* stems

**Table 10: TLC investigation of methanol extract of five plant extracts.**

| No. | Constituent | Solvent System | Plant extract | No. spots | Rf values |
|---|---|---|---|---|---|
| 1 | Anthraglycoside | EtoAc:MeOH:water (100:13.5:10) | E.C | -- | -- |
| | | | E.A | -- | -- |
| | | | E.I | 1 | 0.62 |
| | | | PL | 1 | 0.58 |
| | | | PS | 3 | 0.2<br>0.42<br>0.56 |
| 2 | Bitter principles | EtoAc:MeOH:water (100:13.5:10) | E.C | 2 | 0.05<br>0.69 |
| | | | E.A | 2 | 0.072<br>0.73 |
| | | | E.I | 2 | 0,53<br>0.8 |
| | | | PL | -- | -- |
| | | | PS | 3 | 0.29<br>0.56<br>0.87 |
| 3 | Flavonoid | EtOAc:formica.:glacial acetica.:water(100:11:11:26) | E.C | -- | -- |
| | | | E.A | 1 | 0.68 |
| | | | E.I | 2 | 0.6<br>0.72 |
| | | | PL | 1 | 0.52 |
| | | | PS | 1 | 0.52 |
| 4 | Saponins | $CHCL_3$:glacial acetic a.: MeOH:water(64:32:12:8) | E.C | 1 | 0.14 |
| | | | E.A | 1 | 0.14 |
| | | | E.I | 1 | 0.5 |
| | | | PL | 2 | 0.12<br>0.46 |
| | | | PS | 2 | 0.3<br>0.4 |
| 5 | Coumarins | Diethyl ether:toluene (1:1) | E.C | 1 | 0.59 |
| | | | E.A | 1 | 0.70 |
| | | | E.I | -- | --- |
| | | | PL | -- | --- |
| | | | PS | 3 | 0.14<br>0.63<br>0.73 |

*TLC: thin layer chromatography,* EtoAc: ethyle acetate, MeOH: methanol, CHCL3:chloroform, a: acid, RF: retention factor,---:absent, *EC:Euphorbia cactus Ehrenb, EA:Euphorbia ammak, EI=Euphorbia inerticulata shwenf , PL:pergularia tomentosa L.leaves,, PS:Pergularia tomentosa L. stem*

TLC: (a) anth (anthraquinones) after sprayed with 10%ethanolic KOH agent, (b) BIT(bitter principles) after sprayed with vanillin sulpharic acid, (c) Fl(flavonoids) after sprayed with polythlene glycol, (d) saponins after sprayed with vanillin supharic acid ), and(e) coumarins after sprayed with 10 % ethanolic KOH. Abbreviations at the end of plates in each plateare:PL, *Pergularia tomentosa L* leaves, PS*: Pergularia tomentosa L* stems, EA*: Euphorbia ammak, EI: Euphorbia inerticulta, EC:Euphorbia cactus*