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Antipromastigote activity of *Nicotiana tabacum* Aganist *Leishmania tropica*

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Abstract
Leishmniasis one of the recognized disease in tropical countries including Afghanistan, this research determine the antileishmanial activity of *Nicotiana tabaccum* methanol extracts against promastigotes of *L. tropica*, which is the causative agent of Nangarhar province Afghanistan. Different concentrations (100 µgmL-1, 50µgmL-1, 25µgmL-1) of *Nicotina Tabacum* extraction were screened to investigate *leishmanial* activates against *Nicotiana tabaccum* methanol extracts a long three times (24, 48, 72) Hours. The Percentage inhibition of Promastigote *More* Then (22%) After 24 And 48 Hours incubation, While After 72hours increase (36%). Standard division at the Highest Concentration of 100 µgmL-1 was (0, 094624) While at the Lowest Concentration of 25µgmL-1 was (0.014266) % after 24, 48 And 72 Hours.

Keywords: tobacco, methanol extract, promastigote, inhibition, antileishmanial activity

Introduction
Leishmaniasis is a vector-borne infectious disease that is transmitted by the bite of infected female sand-fly belonging to genus *Phlebotomus*. *Leishmania* is an intracellular obligate protozoan parasite (Dany *et al.*, 2003) [5] and exists in two morphological forms: intracellular amastigotes in phagolysosome of the monocyte-macrophage system and motile promastigotes in the gut of sand-fly and in culture (Rogers *et al*., 2007; Bates, 2007) [14, 3]. *Leishmaniasis* is prevalent in 88 states including Afghanistan, with more than 350 million people having a vulnerability to infection. An estimated frequency of 2 million fresh infections per year occurs in the world (Oryan *et al.*, 2016) [13]. Above ninety percent of CL cases occur in Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia and Syria (WHO, *2017).* There are four species for CL in the Old World which includes L. infantum, L. tropica, L. major and L. aethiopica. In Pakistan, CL is either caused by L. major (zoonotic) or *L. tropica* (anthroponotic) (Bari, 2012; Bauer *et al.*, 1997) [1]. Drugs used for the treatment of CL have high toxicity, which is still challenging in the therapeutic and management process. The first line drugs for CL are sodium stibogluconate, antimony-N-methyl-glutamine, and second line drugs are pentamidine, edelfosine, perifosine, miltefosine, ilmofosine, sitamaquine, paromomycin, amphotericin-B, dapsone, ketoconazole, itraconazole, fluconazole and allupurinol (Berman, 1997; Hepburn, 2001; Jaffar, 2006; Tiuman *et al*., 2011) [4, 7, 9, 16]. The use of plants and herbs for therapeutic purposes is as ancient as human knowledge about the diseases is. Phototherapy is the oldest form of medical treatment e.g., Hippocrates (460 B.C. – 377 B.C), the Greek naturopath and doctors, was interested in plants and have used them for remedy (Ferreira *et al.*, 2014) [6]. Over 200,000 Medicinal plants are known as a rich source of phytomedicans. The use of medicinal plants extracts in traditional medicine is being revisited in modern medicine to find new therapeutic agents (Muthamilselvan *et al.*, 2016) [11].

Material and Methods
Experimental Methodology
The study has been conducted at *Leishmania* and Malaria directorate Nangarhar Province Afghanistan. The materials needed for the study would be RPMI-1640 medium, culture flasks, china dishes, separating funnel, electric balance, 96-well plates, centrifuge machine, dried leaves of *Nicotiana tabaccum* (Tobacco) plant and reagents. The chemicals used as a solvent for fractionation of extracts will include ethyl acetate, n-hexane and chloroform on the basis of polarity.

Plants leaves and their Extraction
Air-dried leaves of *Nicotiana tabaccum* and Camellia sinensis will be ground to fine powder. The powdered sample will then be soaked in commercial grade methanol (97%) for 7-10 days at room temperature. The extracts will then be filtered through Whatman No.2 filter paper. Extraction will be performed by mixing of plant material with 97% methanol at ratio 1:4. I.e.1 gram of powder will be dissolved in 4 mL of methanol. Further extraction from crude extracts will be performed with approximately 1 L of the solvents n-hexane, chloroform, ethyl acetate using the separating funnel, until a clear extract is obtained (Silvia *et al*., 2013; Hong *et al*., 2015) [15, 8]. The solution will be filtered through Whitman No.2 filter paper. The resulting crude extract will be stored at 40C until further analysis.

Culturing *Leishmania tropica* Promastigotes
The Leishmania promastigotes already cryopreserved in Liquid Nitrogen container will be cultured in a flask containing 5-10 mL RPMI-1640 growth medium supplemented with fetal bovine serum (FBS) (10%), antibiotics including Penicillin (100 µgmL-1), Streptomycin (100 µgmL-1) and Hemin (5µgmL-1). The flask will be kept in an incubator at 240C-260C and the medium will be checked and changed on every 2nd, 3rd or 4th day. The time span required for conversion of promastigotes into the metacyclic stage is from 7-10 days. After that the culture will be pouring into centrifuge tubes and then spun at 2000 rpm for 10 minutes. Then the supernatant which form will

pour off carefully from the tubes leaving the pellet only. The pellet will be re-suspended 5-10mL in RPMI-1640 growth medium and thus new cultures will be established.

Testing extracts against *Leishmania tropica* promastigote
The promastigotes will be counted using a Neubauer Hemocytometer and will be added to the 96-well plate at a concentration of 1x105promastigotes/well in 200µL fresh growth medium. To evaluate the anti-promastigotes activity different extracts concentrations i.e.100 µgmL-1, 50µgmL-1, 25µgmL-1 will be added. The plate will be incubated at 260C for the duration of 72 hours in which to observed the effect of these agents after 24, 48 and 72 hours through counting the number of promastigotes microscopically by using Neubauer Haemocytometer.

Results
The percentage inhibition of the *Leishmania* promastigotes by incubation with various extracts will be calculated by using the following formula:
% inhibition =

$$\frac{\text{(Promastigotes in control–Promastigotes in drug treated wells)} \times 100}{\text{Promastigotes in control}}$$

Different concentrations (100 µgmL-1, 50µgmL-1, 25µgmL1) of *Nicotina Tabacum* extraction were screened to investigate *leishmanial* activates against promastigote stage of *leishmania tropica* a long three times of follow up (24, 48, 72) Hours. Phytochemical analysis of methanol extract nicotine tobacco leaf respectively table1. The Percentage inhibition of Promastigote *More* Then (22%) After 24 And 48 Hours incubation, While After 72hours increase (36%). Standard division at the Highest Concentration of 100 µgmL-1 was (0, 094624) While at the Lowest Concentration of 25µgmL-1 was (0.014266) % after 24, 48 And 72 Hours. Respectively Figure 1.

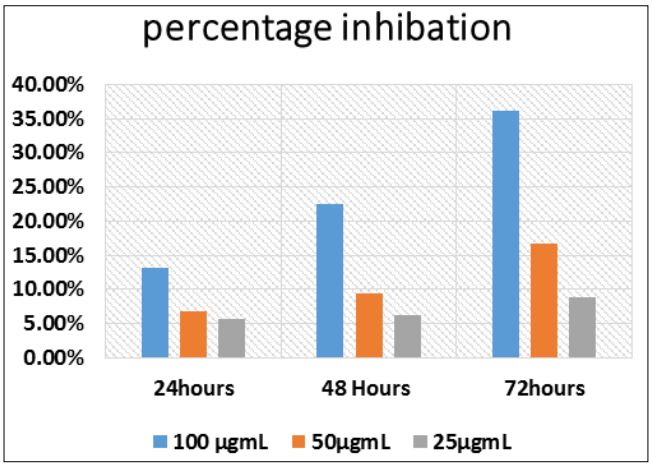

Fig 1: Percentage inhibition against promastigote stage of *leishmana tropica* a long three times (24, 48, 72) Hours.

Table 1: The phytochemical analysis of *Nicotinea Tobaccum* leaf methanol extract.

Phytochemicals	Result
alkaloids	+
flavonoids	+
saponins	+
cardiac glycosides	+
terpenes steroids	+
Balsam	+

Discussion
Nicotina Tabacum methanol extract showed varies antimicrobial activities against promastigote stage of *leishmani tropica.* The highest inhibition percentage obtained from 100 µgmL-1, while the lower concentration of extract 25µgmL-1 showed less inhibitory effects. Extract obtained from tobacco leaves had an antimicrobial effects on *streptococcus pyogenic* and hence recorded high percentage inhibition against the test organism in the agar diffusion essay, the largest percentage inhibition was obtained from methanol extract, tobacco leaf extract showed a little anti-fungal activities against *candida albinos*, in the agar diffusion essay, only the methanol extract showed significant percentage inhibition (Okorondu, *et al*, 2015) [14].

Conclusion and Recommendations
Tobacco has a long history of being used as activities, phytochemical analysis of tobacco leaves showed alkaloids, terpenes steroids, flavonoids, cardiac glycosides and sapiens. Tobacco leaves have great antimicrobial potential, it is possible that natural extract would have lower human toxicity and hence can be used as Medical practitioners in the treatment of various microbial Growth inhibitor of protozoa infection The antimicrobial activates of tobacco already being exploited illness, it is therefore recommended that further research could be carried out to determine the active phytochemical components and purify them for use as novel antimicrobial drugs.

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