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ANTIBACTERIAL ACTIVITY, PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF STEM OF *NICOTIANA TABACUM*

Y. Sharma [1], D. Dua [1], A. Nagar [2] and N. S. Srivastava [*1]

Amity Institute of Biotechnology [1], Amity University, Uttar Pradesh, India,
Helix Bio Genesis Pvt. Ltd. [2], Noida, Uttar Pradesh, India.

Keywords:

Antibacterial activity, Phytochemical screening, *Nicotiana tabacum*, Total flavonoid count and Antioxidant activity

Correspondence to Author:
Dr. Nupur Sinha Srivastava

J-3 Block, Cell line metabolism Lab, Amity Institute of Biotechnology, Amity University, Uttar Pradesh, Noida, Sector -125, India.

Email: nsinha@amity.edu

ABSTRACT: The parts of the *Nicotiana tabacum* plant have been known to possess a wide range of biological activities. The purpose of the present study was to investigate the antibacterial activity, phytochemical screening and the antioxidant activity of aqueous and alcoholic extracts of the stem of *Nicotiana tabacum*. The antibacterial activity was observed against two gram positive bacteria (*Bacillus amyloiquefaciens*, *Staphylococcus aureus*) & two gram negative bacteria *(Escherichia coli*, *Pseudomonas aeruginosa*) in its aqueous, ethanolic, acetone and methanolic extracts by agar well diffusion method where maximum antibacterial activity found to be present in the methanolic and ethanolic extract against *Staphylococcus aureus* with an inhibition length of 10.667±1.527 mm and 8±1.00 mm, respectively. Phytochemical screening of aqueous, ethanolic & methanolic extract revealed the presence of saponin, flavonoids and alkaloids. Flavonoid content in stem was found to be 838 mg QE/g of extract in the 80% of ethanol extract by aluminium chloride colometric method. Antioxidant activity was observed in order to estimate the superoxide dismutase, catalase, glutathione content, glutathione s transferase & lipid peroxidation i.e. Malondialdehyde (MDA) content in the aqueous and methanolic extract where methanolic extract has shown a high level of antioxidant activity. The present study suggests that the stems of *Nicotiana tabacum* can be as a good antimicrobial and antioxidant agent.

INTRODUCTION: Natural bioactive compounds have shown various anti-bacterial, anti-fungal, and anti-inflammatory properties. They are gaining considerable attention as eco-friendly alternative to synthetic antibacterial active compound or agents [1]. There are a huge number of herbal medicines described in Ayurveda and other alternative traditional medicines whose utilization in general health of the common people is still not efficient because of several reasons that is secondary metabolites which posses several properties.

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The medicinal plants are being utilized by human beings in universal phenomena. The World Health Organization has recognized the important role of medicinal plant in health care, where 80% of the world Population is dependent on traditional medicine [2]. It has also been reported that different parts of a particular plant consist of different secondary metabolites and different antibacterial properties that has been a counterfeit to know which part should be used for therapeutic agent [3].

For years plants have been a valuable source of natural products for maintaining human health. Researchers have already used the extracts of plants for various anti-bacterial, anti-fungal and anti-viral activities [4]. The waste product of plants has also shown the presence of bioactive compounds such as stem or bark. Phytochemicals have reveals the active component of medicinal

Plant that has shown effect against on all types of microorganism and also their sensitive test against Gram positive bacteria & gram negative bacteria [5]. As far as the free radicals are concerned, antioxidant activity provides medical revolution for health and disease management [6]. There are many medicinal plants that have shown various antioxidant activities against the stress provided by the 6 hydroxydopamine [7]. Antioxidant component have the ability for scavenging free radicals which can damage several protein and DNA, leading to genomic instability and cancer [8]. As far as the flavonoids are concerned, these polyphenolic flavones are responsible for various activities such as anti-bacterial, anti-inflammatory, anti-oxidants and many others [9]. The antioxidant activity depends upon its molecular structure where the position of hydroxyl group and other features have shown maximum importance for their antioxidant activity [10, 11].

Nicotiana tabacum (Tobacco) belongs to a family of Solanaceae. It's a perennial herbaceous plant that is found only in cultivation, it grows up to 2 meters in height. It is native to tropical and subtropical America but today it is cultivated throughout the world. All the parts are sticky and are covered by shorts viscid-glandular hairs which exude a yellow secretion containing nicotine. Its synonyms are tobacco, tamak and siah (marma). 20% of tobacco resources are discarded as processing waste, which pollutes the environment and cause a big waste [12]. In India, the leaves of tobacco plant have been used as sedative, antispasmodic, vermifuge, antiseptic, emectic and nacrotic.

The decoction of leaves also applied for muscle relaxation and relieving pain [13]. Discarded tobacco leaves are valuable because of the presence of bioactive compound [1]. However, tobacco leaf is rich in polyphenols which posses various bioactive that affect the quality of tobacco leaf [14, 15]. Nicotine, which is isolated from leaves of tobacco in associate with zinc has shown the antibacterial activity against ten different strains of gram positive and gram negative bacterial strain [13]. The ant-inociceptive activities of methanolic leaf extract of tobacco using by tail immersion, hot plate and acetic acid has revealed the abdominal constrictions in albino Wistar mice [16]. Tobacco has also known for its antifungal activity against *Fusarium solani* [17]. As a traditional medicine, for the treatment of tuberculosis and coughs were also screened for activity against *Mycobacterium tuberculosis* [18].

Tobaccos contain 30% - 40% of vegetal oil and are able to produce oil and biodiesel. Tobacco also consists of citric acid that can be used for the production of dye and varnishes. As far as the stem of tobacco is concerned, production of briquettes has values of tobacco stem which consist of only minimum quantity of Nicotine i.e. 0.005% [19]. It has been reported that extracts of seeds have shown antibacterial activity against *Staphylococcus*. [20]. The antioxidant activity of tobacco leaves also has shown that flavonoids of tobacco possess superoxide anion, DPPH and ABTS radical scavenging abilities to ascorbic acid at high concentration, i.e. 600µg/ml [21]. Young and adult plant parts of tobacco from Tunisia have also shown antioxidant activity dried at different temperature i.e. 40 and 70 °C. In tobacco leaves generally had higher amount of phenol i.e. 14.46-23.05 mg GAE g^{-1} [22]. The tobacco plant extract also reported to be the Anti Alzheimer's activity where its plant extracts improved memory [12].

The purpose of present study was to investigate the antibacterial activity of different extracts of the stem of *Nicotiana tabacum* against different bacterial cells by an agar well diffusion method. Phytochemical screening was done in order to reveal the presence of secondary metabolites in its aqueous and alcoholic extracts of the stem of *Nicotiana tabacum*. The total flavonoid content was done to estimate the quantity of flavonoids in the extract by aluminium chloride colometric method.

The antioxidant activity of aqueous and alcoholic extracts of the stem of *Nicotiana tabacum* was observed to determine the free radical scavenging capacity of flavonoids that were present in the following extracts. These experiments were initialized to provide a scientific rationale for the use of stem as a traditional herbal remedy which was being discarded as a waste by human being.

MATERIALS AND METHODS:

Plant Samples: The plants of *Nicotiana tabacum* were collected from Khari Baoli, Kucha Challan, Chandni Chowk, Delhi and kept at Amity Institute of Biotechnology, Amity University Uttar Pradesh. The plant was identified by Dr. Nupur Sinha Srivastava. Stems were separated out from the leaves, were then washed with distilled water to remove dirt and soil particles. These stems were dried in the shaded area at room temperature for a period of one week. The dried stems were grounded with an ordinary grinder to form a powder and used throughout the project.

Bacterial Strains:

The bacterial strains of *Escherichia coli* (DH5α), *Bacillus amyloliquefaciens, Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from Helix BioGenesis Pvt. Ltd., Noida, U.P. They were sub cultured freshly in Luria Broth (LB) medium and used further for research work.

Preparation of Antibacterial Extracts:

Aqueous extract and alcoholic extracts were prepared after washing, drying and crushing of stems of *Nicotiana tabacum* by ordinary grinder to make fine powder. The 5 g of stem crushed powder was dissolved in 50 ml of distilled water i.e. Aqueous extract and different solvents such as acetone, ethanol & methanol in order to prepare alcoholic extract and it was boiled in water bath for aqueous extract about 30 minutes at 100°C and for alcoholic extract at 65°C. The conical flasks of the extract were covered by cotton plugs to avoid the evaporation. The extracts were placed in shaking incubator at 250 rpm for 24 hrs. After shaking they were filtered with muslin clothes and again filtered with filter paper twice. The filtered aqueous extract was stored at 4°C. Whereas alcoholic extracts were evaporated to dryness and extract amount were measured [23].

Antibacterial Sensitive Test:

Agar well diffusion method:

LB agar media were prepared and autoclaved at 121°C for 15 minutes at 15 Lbs and poured in sterile petri plates up to a uniform thickness of approximately 10 - 15 minutes and the agar was allowed to set at ambient temperature. This method is suitable for the organism to grow rapidly overnight at 35 - 37°C. The wells were made in medium after inoculation with the microorganism. 200 µl of inoculums were spread over LB agar plates using sterile spreader, after few minutes four wells were made in each Petri plated and loaded with 100 µl of extracts, control and standard antibiotics. Plates were incubated at 37°C for 24 hrs. Antibacterial activity was observed by measuring its inhibition length. Inhibition length against bacteria was calculated by as previously described [3, 23]. The experiments were done in triplicate.

Inhibition length = Zone of Inhibition (mm) – Well diameter (mm)

Preliminary Phytochemical Screening:

Preliminary phytochemical screening was done by standardized protocol of [23]. For this dried stems were crushed with Water, ethanol and methanol approximately 30 ml extracts were prepared by boiling in hot water bath for at half an hour and passing by the same through muslin cloth for filtrations [23]. Phytochemical screening was quantified in order to reveal the presence of secondary metabolites such as saponin, tannin, flavonoid, terpenoid, napthoquinone, inulin, carbohydrate, alkaloid and phenol in the aqueous, ethanolic and methanolic extracts of the stem of *Nicotiana tabacum*.

Determination of Flavonoids:

For the determination of total flavonoid content in 80% ethanol extract was determined by Aluminium chloride colorimetric method [11]. Maceration extracting method was used. 0.5 ml of extract (1mg/ml) or Quercetin standard (5 to 40µg/ml), 1.5ml methanol, 0.1ml aluminium chloride, 0.1ml potassium acetate solution and 2.8ml distilled water were added and mixed well. Sample blank was prepared in a similar way by replacing aluminium chloride with distilled water and absorbance was measured at 430 nm. The standard calibration curve was made to determine the concentration of flavonoid in the extract. The results were expressed in mg Quercetin Equivalent g^{-1} [11].

Antioxidant activity:

Antioxidant activity was determined by enzymatic and non-enzymatic biochemical assay. The

superoxide dismutase (SOD) activity, catalase (CAT) activity, glutathione content (GSH), and glutathione s transferase (GST) activity were observed in aqueous and methanolic extract of the stem of *Nicotiana tabacum* by standard protocol [7]. Superoxide dismutase activity was expressed as one unit of enzyme activity was defined as the enzyme concentration required for inhibition of the absorbance at 560nm of chromogen production by 50% in 1 min under assay conditions and expressed as specific activity in the unit of SOD per min per mg of protein [24]. Catalase activity was expressed in enzyme activity as µmoles of H_2O_2 oxidized per min per mg protein [25].

Lipid peroxidation i.e. thiobarbituric acid reactive substances were measured spectrophotometrically at 532nm in aqueous and methanolic extracts of the stem of *Nicotiana tabacum* on the principle of formation of Malondialdehyde (MDA) by breaking down of polyunsaturated fatty acids where the levels of lipid peroxidation were expressed as nano mole of malondialdehyde formed per g of tissue [26]. Estimation of GSH content in the aqueous and methanolic extracts of the stem of *Nicotiana tabacum* was done by using dithiobis nitrobenzoic acid (DTNB) and expressed in µg per mg of protein [27]. Glutathione-s-transferase (GST) activity was measured in the aqueous and alcoholic extracts of the stem of *Nicotiana tabacum*. The GST assay was based on glutathione conjugation to 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate and measured spectrophotometrically at 340 nm. The specific activity of the enzyme was expressed as µmole of CDNB-GSH conjugate formed per min per mg protein [28].

Rapid screening of antioxidant compound in aqueous and amethanolic extracts of the stem of *Nicotiana tabacum* was done by Dot blot and DPPH staining. Each dilutes extract was loaded on a TLC layer and allowed to dry for 3 min. The drop of each extract was loaded simultaneously in increasing concentration (10 and 20 µg/ml) the sheet bearing the dry spot were placed upside down for 10 s in 4 mM DPPH. A purple background was revealed by stained silica layer with a white spot at the location where radical scavenging was observed in the aqueous and methanolic extracts of the stem of *Nicotiana tabacum* [29].

Units:

SOD: Unit/min/mg protein, CAT: µmoles of H_2O_2 consumed/min/mg protein, Glutathione content (GSH): µg of glutathione /mg protein, GST: µmoles of CDNB-GSH conjugate formed/ min/mg protein and Lipid peroxidation: MDA content in µmoles /mg protein sample.

Quantification of Protein:

Protein was quantified in the 100µl supernatant by Lowry's method by using Floin Ciocalteau Reagent [30].

Statistical Evaluation:

To assure the accuracy of the experimental data, each experiments were performed in triplicate and the result was expressed as mean ± standard deviation of three replications. *P* value < 0.05 was regarded as significant.

RESULTS:

Antibacterial Screening Test:

The purpose of the present study was to investigate the antibacterial activity of different extracts of stem of the tobacco plant by agar well diffusion method. Inhibition length was calculated in order to reveal its inhibitory effect against two gram positive (*Bacillus amyloiquefaciens*, *Staphylococcus aureus*) and two gram negative bacteria *(Escherichia coli*, *Pseudomonas aeruginosa*) as shown in **Fig. 1** and its antibacterial activity compared with standard antibiotics such as streptomycin (**Table 5**). From the **Graph 1**, this was observed that maximum antibacterial activity was found to be present in the methanolic and ethanolic extract of the stem of *Nicotiana tabacum* against *Staphylococcus aureus* with an inhibition length of 10.667±1.527 mm and 8±1.00 mm, respectively as compared to standard.

Methanol extracts of the stem of *Nicotiana tabacum* have shown an inhibitory effect against *Pseudomonas aeruginosa* with an inhibition length of 5.33±1.154 mm (**Table 2**). As compared to the aqueous extract, it has shown maximum antibacterial activity against *Staphylococcus aureus* with an inhibition length of 4.667±1.154 mm (**Table 1**). Ethanolic extract has also shown its inhibitory effect against *Bacillus amyloliquefaciens* with an inhibition length of 4.667±1.154 mm

(**Table 3**). The inhibitory effect was also found to be present in the acetone extract against *Bacillus amyloliquefacien*, *Staphylococcus aureus* and *Escherichia coli* with an inhibition length of 1.33±0.577 mm, 4.33±1.154 mm and 2±0.00 mm, respectively (**Table 4**). Growth of *Escherichia coli* found to be inhibited by the aqueous, ethanol and methanol extract of the stem of *Nicotiana tabacum* against *Escherichia coli* with an inhibition length of 1.33±1.527 mm, 1.667±0.577 mm and 1.667±0.577 mm, respectively. Acetone extracts have also shown its inhibitory effect against *Pseudomonas aeruginosa* with an inhibition length of 2±0.00 mm (**Table 4**).

The growth of *Pseudomonas aeruginosa* was also found to be inhibited by aqueous and ethanolic extract with an inhibition length of 1.33±1.527 mm and 1±0.00 mm, respectively (**Table 3**). Aqueous extract has shown a less inhibitory effect against *Bacillus amyloliquefaciens* with an inhibition length of 0.66±0.577 mm where as methanolic extract has shown inhibitory effect against 4±1.00 mm. In contrast to streptomycin, it was observed that antibacterial activity was found to be present maximum in methanolic and ethanolic extract of the stem of *Nicotiana tabacum* against *Staphylococcus aureus.*

Preliminary Phytochemical Screening:
Phytochemical screening was done in order to reveal the presence of secondary metabolites that were present in the different extracts of the stem of *Nicotiana tabacum*. Saponin, flavonoid, terpenoid, alkaloid and inulin found to be present in the aqueous extract where as tannin, napthoquinine, carbohydrate and phenol found to be absent. Saponin, flavonoid and alkaloid found to be present in the ethanolic and methanolic extract of the stem of *Nicotiana tabacum* where as tannin, terpenoid, napthoquinone, inulin, carbohydrate and phenol, found to be absent in the ethanol and methanol of the stem of *Nicotiana tabacum* (**Table 6**).

Quantification of Flavonoids: Total flavonoid content was done in order to estimate the number of flavones in the ethanol extracts of the stem of *Nicotiana tabacum*. Quantification of flavonoid was done by the aluminium chloride colometric method. By using a standard plot of quercetin (y = 0.012x, R^2 = 0. 963) as shown in **Graph 3**. The flavonoid content of the stem of *Nicotiana tabacum* was found 838 mg QE/g of extract in the 80% of ethanolic extract.

Antioxidant activity:
Antioxidant activity was observed in aqueous and methanolic extract of the stem of *Nicotiana tabacum* with respect to the specific enzyme activity of those flavonoids that were observed in its ethanolic extract. The superoxide radical scavenging effects of methanolic extract were analyzed in the terms of formazan and SOD activity which was calculated in terms of unit/mg of protein. SOD activity in the methanolic and aqueous extract of the stem of *Nicotiana tabacum* found to be 4.05±0.104 Unit/min/mg protein and 2.866±0.152 Unit/min/mg protein. Higher level of SOD activity in methanol extracts demonstrates the powerful superoxide anion scavenger that can used as a therapeutic against oxidative stress as shown in **Graph 2**.

The catalase activity found to be present in both the extracts by scavenging activity of hydrogen peroxide. Methanolic extract and aqueous extract has shown 3.263±0.227 µmoles of H_2O_2 consumed/min/mg protein and 2.226±0.289 µmoles of H_2O_2 consumed/min/mg protein, respectively. Glutathione content (GSH) was also found to be present in the aqueous and methanolic extracts of the stem of *Nicotiana tabacum* where as it has shown a high level in the methanolic extract i.e. 11.056±0.486µg of glutathione oxidized/mg protein. Aqueous extract of the stem of *Nicotiana tabacum* has also shown the glutathione content i.e. 7.89±0.845 µg of glutathione oxidized/mg protein. In this study it was also observed that the methanol extract revealed the highest level of glutathione content activity, then aqueous extract as shown in **Graph 2**.

The aqueous extract of the stem of *Nicotiana tabacum* has shown maximum glutathione s transferase activity i.e. 13.017±0.525 µmoles of CDNB-GSH conjugate formed/ min/mg protein where as methanolic extract has shown 8.569±0.521 µmoles of CDNB-GSH conjugate formed/ min/mg protein **Graph 2**.

Lipid peroxidation activity was also observed in the aqueous and methanolic extract and as per the study aqueous extract has shown a maximum level of malondialdehyde (MDA) i.e. 8.471±0.50 µmoles/mg protein sample as compared to methanol it has shown less malondialdehyde (MDA) 0.161±0.04 µmoles/mg protein sample. The total antioxidant capacity of stem of *Nicotiana tabacum* was further determined by DPPH scan blot assay where methanol extract has shown the maximum antioxidant capacity, which was confirmed by the formation of orange spot against the purple background as shown in **Fig. 2- 3**.

TABLE 1: ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACT OF STEM OF *NICOTIANA TABACUM* AGAINST BACTERIAL STRAIN.

Bacterial strain	Well diameter (mm)	Zone of Inhibition (mm)	Inhibition length (mm)
Bacillus amyloliquefaciens	9	9.66±0.577	0.66±0.577
Staphylococcus aureus	9	13.667±0.57735	4.667±0.577
Escherichia coli	9	10.33±1.527	1.33±1.527
Pseudomonas aeruginosa	9	9.6667±0.57735	0.66±0.557

TABLE 2: ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACT OF STEM OF *NICOTIANA TABACUM* AGAINST BACTERIAL STRAIN.

Bacterial strain	Well diameter (mm)	Zone of Inhibition (mm)	Inhibition length (mm)
Bacillus amyloliquefaciens	9	13±1.00	4±1.00
Staphylococcus aureus	9	19.667±1.527	10.667±1.527
Escherichia coli	9	10.667±0.577	1.667±0.577
Pseudomonas aeruginosa	9	14.33±1.154	5.33±1.154

TABLE 3: ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF STEM OF *NICOTIANA TABACUM* AGAINST BACTERIAL STRAIN.

Bacterial strain	Well diameter (mm)	Zone of Inhibition (mm)	Inhibition length (mm)
Bacillus amyloliquefaciens	9	13.667±1.154	4.667±1.154
Staphylococcus aureus	9	17±1.00	8±1.00
Escherichia coli	9	10.667±0.577	1.667±0.577
Pseudomonas aeruginosa	9	10±0.00	1±0.00

TABLE 4: ANTIBACTERIAL ACTIVITY OF ACETONE EXTRACT OF STEM OF *NICOTIANA TABACUM* AGAINST BACTERIAL STRAIN.

Bacterial strain	Well diameter (mm)	Zone of Inhibition (mm)	Inhibition length (mm)
Bacillus amyloliquefaciens	9	10.33±0.577	1.33±0.577
Staphylococcus aureus	9	13.33±1.154	4.33±1.154
Escherichia coli	9	11±0.00	2±0.00
Pseudomonas aeruginosa	9	11±0.00	2±0.00

TABLE 5: ANTIBACTERIAL ACTIVITY OF STREPTOMYCIN AGAINST BACTERIAL STRAIN AS STANDARD.

Bacterial strain	Well diameter (mm)	Zone of Inhibition (mm)	Inhibition length (mm)
Bacillus amyloliquefaciens	9	20±0.00	11±0.00
Staphylococcus aureus	9	25±0.00	16±0.00
Escherichia coli	9	26.3±0.00	17.3±0.00
Pseudomonas aeruginosa	9	24±0.00	15±0.00

TABLE 6: PHYTOCHEMICAL SCREENING OF AQUEOUS, ETHANOLIC AND METHANOLIC EXTRACT OF STEM OF *NICOTIANA TABACUM*.

S/No	Phytoconstituents	Aqueous	Ethanol	Methanol
1	Saponin	+	+	+
2	Tannin	-	-	-
3	Flavonoid	+	+	+

4	Terpenoid	+	-	-
5	Napthoquinone	-	-	-
6	Alkaloid	+	+	+
7	Inulin	+	-	-
8	Carbohydrate	-	-	-
9	Phenol	-	-	-

(+) indicates the presence of the constituents while (–) indicates the absence of constituent

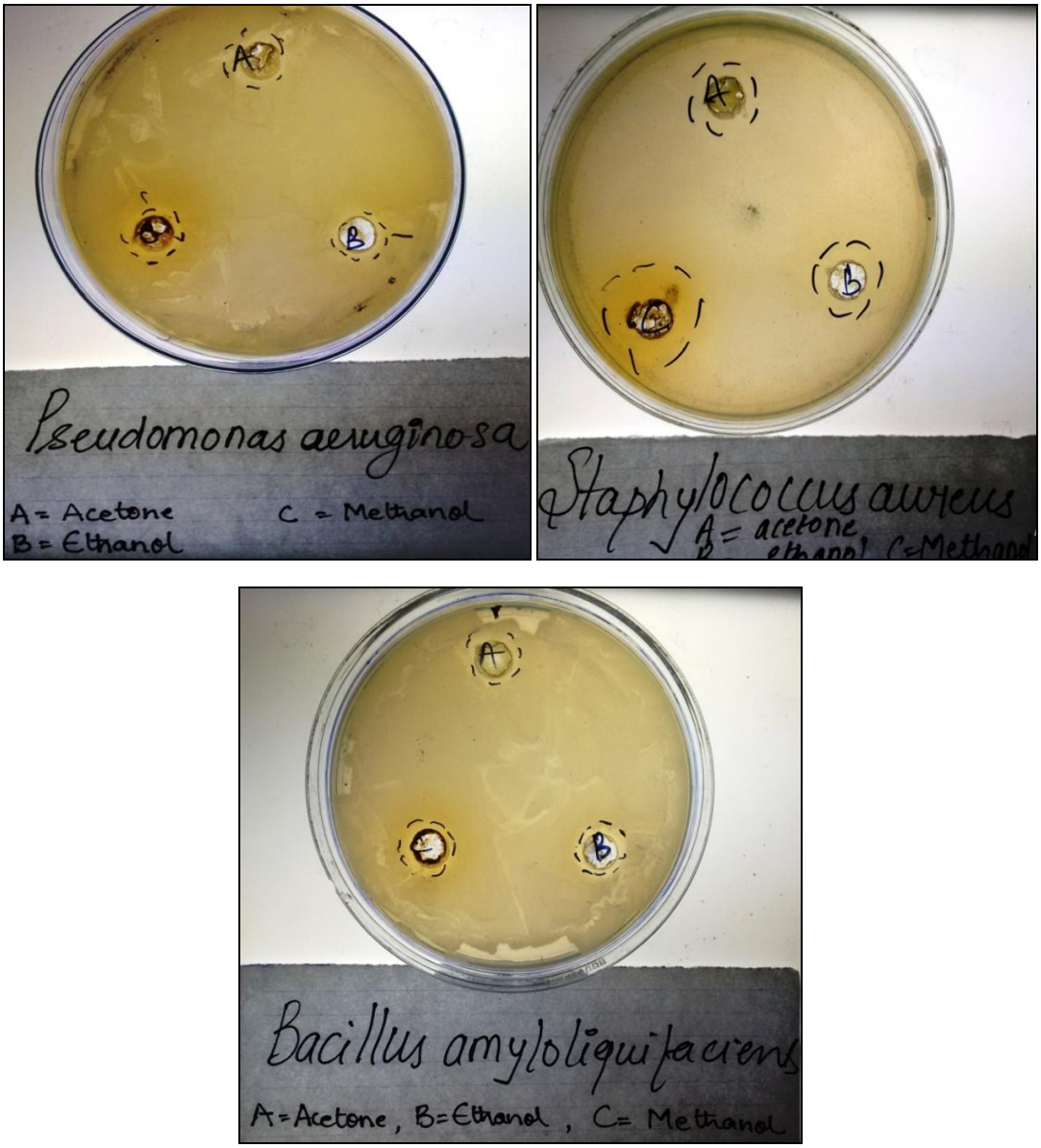


FIG.1: THE ANTIBACTERIAL ACTIVITY OF (A) ACETONE, (B) ETHANOLIC AND (C) METHANOLIC EXTRACT OF STEM OF *NICOTIANA TABACUM*.

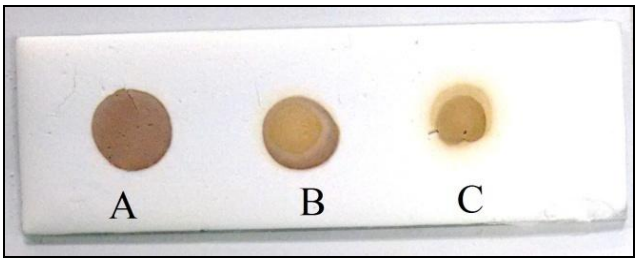


FIG.2: RAPID SCREENING OF ANTIOXIDANT COMPOUND IN AQUEOUS EXTRACTS OF STEM OF *NICOTIANA TABACUM* WHERE (A) 4mM DPPH, (B) 10µG/ML AQUEOUS EXTRACT + 4mM DPPH AND (C) 20 µG/ML AQUEOUS EXTRACT + 4mM DPPH.

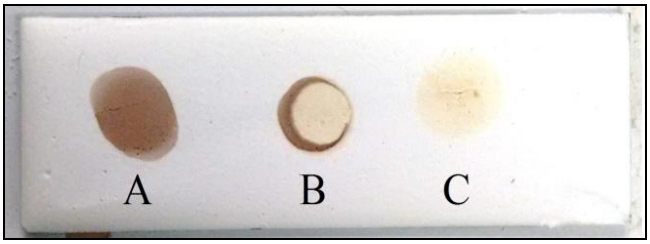
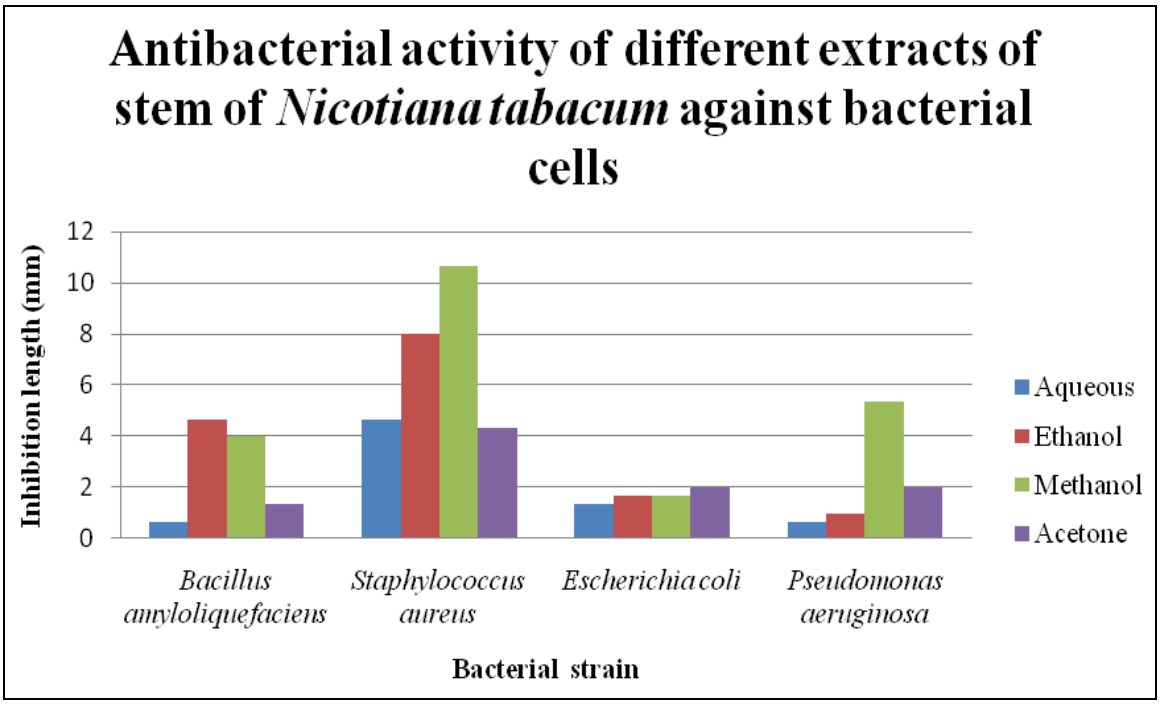
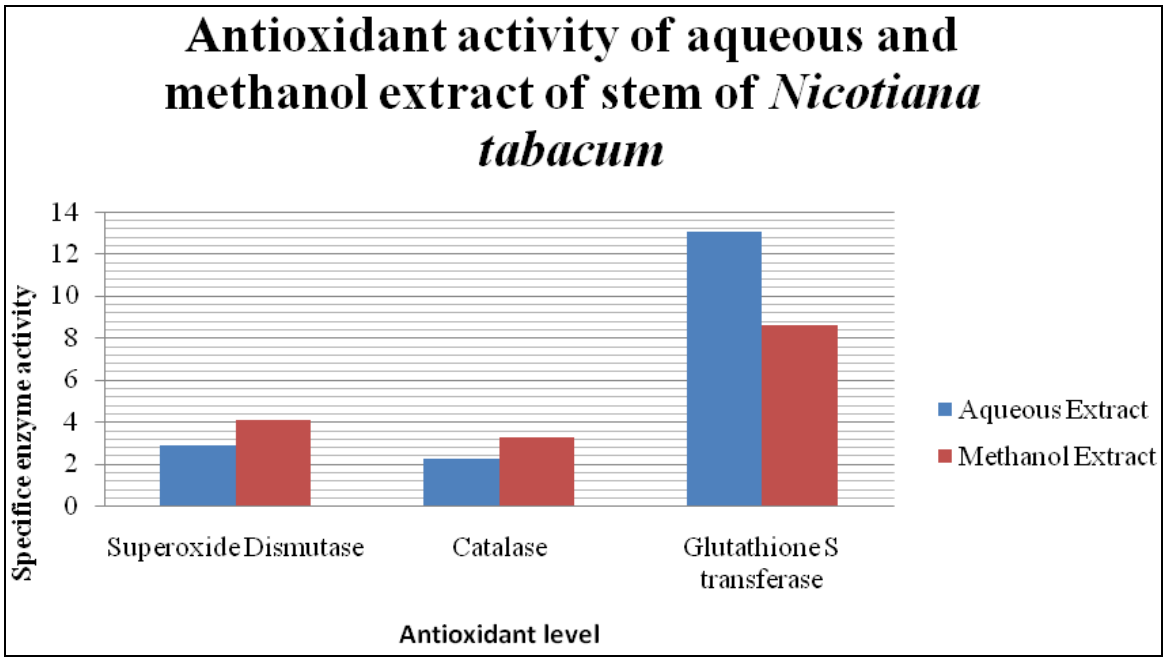
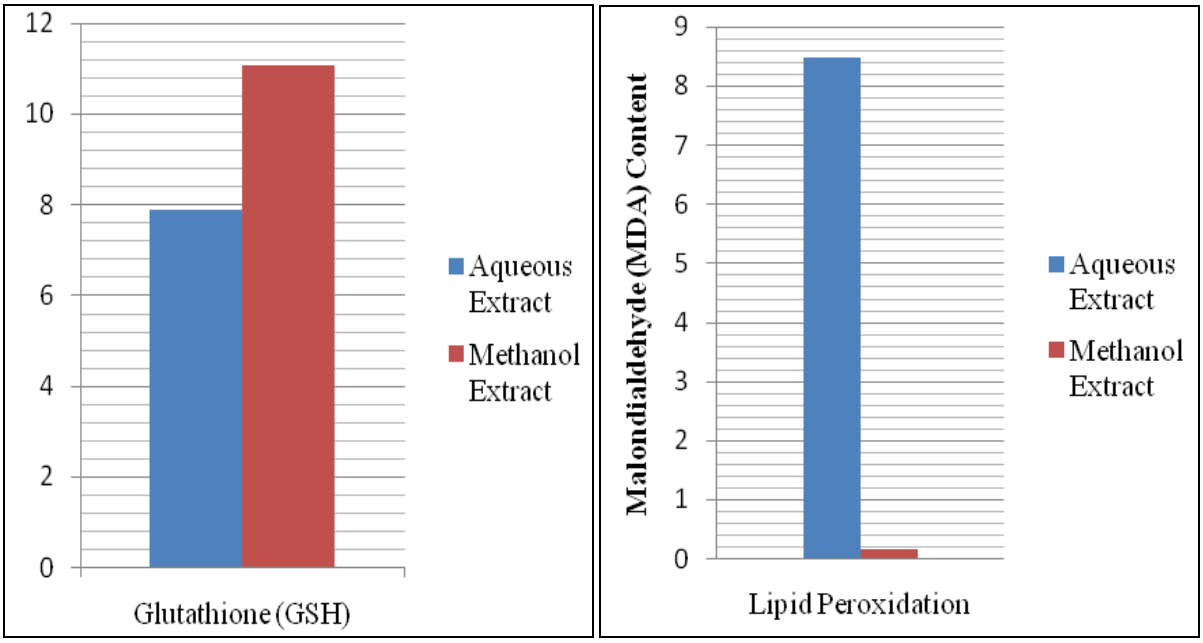


FIG.3: RAPID SCREENING OF ANTIOXIDANT COMPOUND IN METHANOLIC EXTRACTS OF STEM OF *NICOTIANA TABACUM* WHERE (A) 4mM DPPH, (B) 10µG/ML METHANOLIC EXTRACT + 4mM DPPH AND (C) 20 µG/ML METHANOLIC EXTRACT + 4mM DPPH.

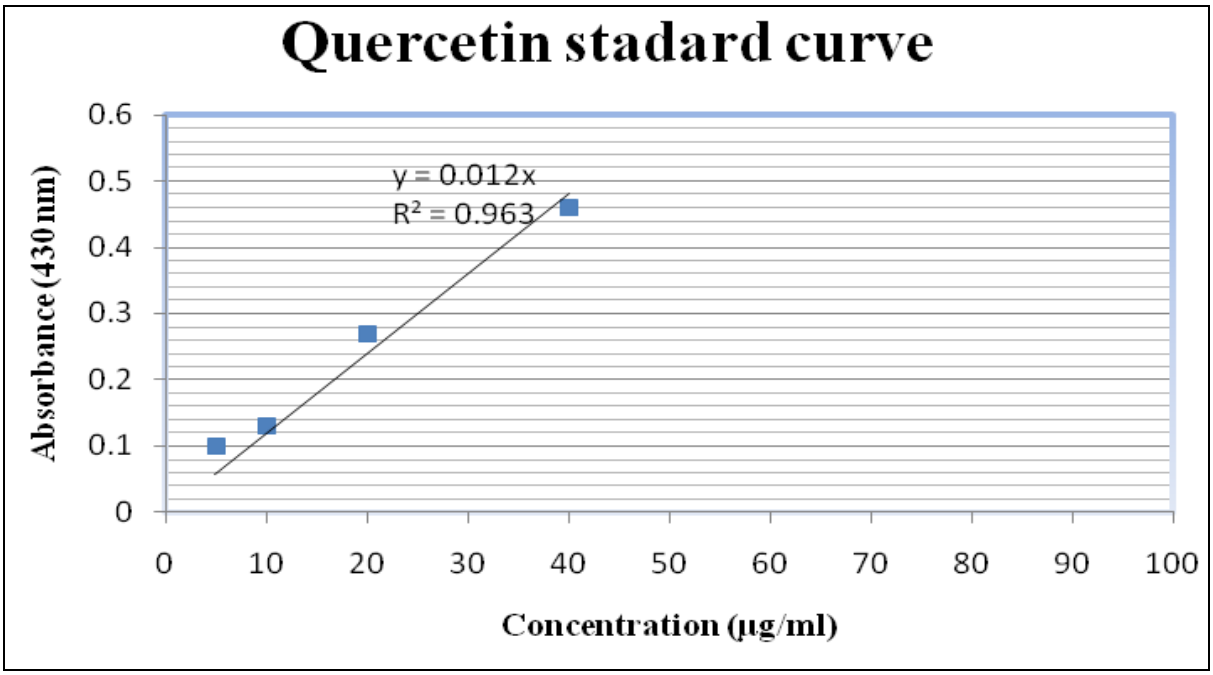


GRAPH 1: ANTIBACTERIAL ACTIVITY OF AQUEOUS, ETHANOLIC, METHANOLIC AND ACETONE EXTRACTS OF STEM OF *NICOTIANA TABACUM* PLANT AGAINST, *BACILLUS AMYLOLIQUEFACIENS, STAPHYLOCOCCUS AUREUS, ESCHERICHIA COLI* AND *PSEUDOMONAS AERUGINOSA*.





GRAPH 2: ANTIOXIDANT ACTIVITY OF AQUEOUS AND METHANOLIC EXTRACTS OF STEM OF *NICOTIANA TABACUM* SHOWING DIFFERENT LEVELS OF FREE RADICAL SCAVENGING.



GRAPH 3: CALIBRATION CURVE OF QUERCETIN STANDARD

DISCUSSION: In Ayurvedic and other alternative traditional medicine, there are numbers of medicinal plant that are being used in curing general health of the common people which is not efficient because of several reasons [12]. It has been previously stated that different parts of plant consist of different antibacterial activity because of their secondary metabolites that are present over there [3]. Flavonoids contributes directly to antibacterial and antioxidant activity due to their scavenging activities confirmed by their hydroxyl group [21]. The following study is an initial screening in the field of microbial activity and antioxidant activity in the stem of *Nicotiana tabacum* the purpose of the study was to investigate the antibacterial activity, phytochemical screening, and antioxidant activity of the stem of *Nicotiana tabacum*. From the above study it has been observed that the maximum amount of antibacterial activity found to be present in the methanolic extract of the stem of *Nicotiana tabacum* against both gram positive bacteria i.e. *Staphylococcus*

aureus with an inhibition length of 10.667±1.527mm and gram negative bacteria against *Pseudomonas aerugionosa* i.e. With an inhibition length of 5.33±1.154mm. As compared to the previous studies leaves of *Nicotiana tabacum* has not shown any activity against *Staphylococcus aureus* in its methanolic extract [31]. Stem of *Nicotiana tabacum* has shown inhibitory effect against *Bacillus amyloliquefaciens*, *Escherichia coli* and *Pseudomonas aeruginsa* with an inhibition length of 4±1.00 mm, 1.667±0.577 mm, and 5.33±1.154 mm in its methanolic extract, respectively.

In contrast to the previous studies no inhibitory effect was found in the methanolic extract of leaves of *Nicotiana tabacum* against *Escherichia coli* [31]. Ethanolic extract of stem of *Nicotiana tabacum* has also shown its antibacterial activity against *Bacillus amyloliquefaciens*, *Escherichia coli* and *Pseudomonas aerugionosa* with an inhibition length of 4.667±1.154 mm, 1.667±0.577 mm, 1±0.00 mm, respectively, where it has given maximum antibacterial activity against *Staphylococcus aureus* with an inhibition length of 8±1.00 mm. As compared to the previous studies ethanolic extract of the leaves of *Nicotiana tabacum* has shown inhibitory effect against *Pseudomonas aeruginosa* with a zone of inhibition i.e. 10.33 mm at 24 conc. mg $disc^{-1}m$ [32] and no inhibitory effect was found against *Escherichia coli* and *Staphylococcus aureus* [32]. The inhibitory effect was also shown in the acetone extract of the stem of *Nicotiana tabacum* where it has shown a maximum inhibitory effect against *Staphylococcus aureus* with an inhibition length of 4.33±1.154 mm whereas, compared to the previous study leaves of *Nicotiana tabacum* has shown maximum antibacterial activity against the same with a zone of inhibition i.e. 8.33mm [32].

Growth of gram positive bacteria (*Bacillus amyloiquifaciens*, *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa)* found to be inhibited by the aqueous extract of the stem of *Nicotiana tabacum* where as inhibitory effect was found against *Eschericia coli* i.e. With a zone of inhibition of 10.66 mm [32]. Preliminary phytochemical screening of aqueous, ethanolic and methanolic extract of the stem of *Nicotiana tabacum* was done in order to reveal the presence of the flavonoid so that the antioxidant activity of aqueous and methanolic extract can be observed. In the previous studies, it was observed that the stem of *Nicotiana tabacum* consist of 0.005% nicotine [19] which signifies that any other secondary metabolites such as flavonoid would be in large amount. For this flavonoid was quantified by the aluminium colometric method in to calculate the number of flavones.

It was observed that 838 mg QE/g of extract found to be present in the 80% of ethanol extract of the stem of *Nicotiana tabacum*. On the basis of the presence of flavonoid, antioxidant activity was observed in the aqueous and methanolic extract of the stem of *Nicotiana tabacum*. It was found from the above study higher level of Glutathione s transferase was found to be present in the aqueous extract i.e. 13.017±0.525 µmoles of CDNB-GSH conjugate formed/min/mg protein with respect to the methanolic extract where the glutathione content (GSH) i.e. 11.056±0.486 µg of glutathione /mg protein found to be high in the stem of *Nicotiana tabacum* as compared to the previous study, curry leaves has shown glutathione peroxidase & glutathione content i.e. 0.5±0.05 µmoles/min/mg protein and 65.4±0.5 µ/mg protein, respectively [7]. High level of superoxide dismutase and Catalase activity found to be present in the methanolic extract of the stem of *Nicotiana tabacum* i.e. 4.05±0.104 unit/min/mg protein and 3.263±0.227 µmoles of H_2O_2 consumed/min/mg protein, respectively. Whereas compared to the methanolic extract of curry leaves, it has shown high level of superoxide dismutase activity i.e. 84.1±0.3 unit/min/mg protein and catalase activity i.e. 1.9±0.05 µmoles of H_2O_2 consumed/min/mg protein, respectively [7].

Lipid peroxidation i.e. Malondialdehyde (MDA) content was also found to be present in the aqueous extract of the stem of *Nicotiana tabacum* i.e. 8.471±0.50 µmoles/mg protein sample where as no malondialdehyde (MDA) content found in the methanol extract of the stem of *Nicotiana tabacum*. Rapid screening for total antioxidant capacity was observed by DPPH scan blot assay in the aqueous and methanolic extract of the stem of *Nicotiana*

tabacum which revealed the high antioxidant capacity in methanolic extract which was confirmed by the formation of orange spot against the purple background.

CONCLUSION: Authors conclude from the above investigation that bioactive extracts of the stem of *Nicotiana tabacum* can be utilized as an active antibacterial agent against microbial diseases. Presence of flavonoid in the preliminary phytochemical screening, flavonoid count and antioxidant activity in the stem of *Nicotiana tabacum* suggests to use it as a natural antioxidant that could have great importance as a therapeutic agent. Future work includes the purification and quantification of secondary metabolites to use stem of *Nicotiana tabacum* as a traditional herbal remedy.

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