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Phytochemical and Antimicrobial Investigation of Indigofera Nummulariifolia (Livera) Ex. Alston (Fabaceae)

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Abstract- *Indigofera nummulariifolia (Livera) Ex. Alston (Fabaceae) is a tropical and subtropical medicinal plant parts reportedly used by ethnic communities treat 'liver complaints'. Phytochemical constituents and antibacterial potentials of Indigofera nummulariifolia (Livera) Ex. Alston (Fabaceae) (whole plant) were investigated. In vitro antibacterial activity using agar-well diffusion method was carried out against Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus subtilis, Candida albicans and Aspergillus flavus. Phytochemical screening revealed the presence in the methanol extract of glycosides, tannins, flavonoids, saponins, cardiac glycosides, steroids/triterpenoids and alkaloids. Cardiac glycosides and steroids/triterpenes tested positive in ethyl acetate extract while steroid/triterpenes and alkaloids were detected in the dichloromethane and n-hexane extracts. Most of the extracts showed antimicrobial activity with zones of inhibition in the range of 15mm and 19mm except for methanol extract with no antifungal activity. Minimum Inhibitory Concentrations of 25mg/mL and Minimum Bactericidal/ Fungicidal Concentrations of 50mg/mL were observed for most extracts against Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus subtilis, Candida albicans and Aspergillus flavus. Ethno-medicinal use of I.nummulariifolia to treat 'liver complaints' may be justified. The plant could be a novel source of antibacterial and antifungal agent (s) with possible broad spectrum activity*

Key Words: *antimicrobial, ethno-medicinal, Ingigofera nummulariifolia, liver complaints, minimum bactericidal /fungicidal concentration, minimum inhibitory concentration, phytochemical, zones of inhibition*

1.INTRODUCTION

Long before the emergence of modern medicine traditional medical practices grew for centuries as beliefs and understandings of sicknesses kept changing with increasing experience, observation and enlightenment transmitted from generation to generation. However what projects the status of the traditional medical practitioner the most is his involvement with medicinal plants. The use of plant materials (leaves, roots, stems, flowers, seeds or whole plant) as useful sources of medicines in traditional medical practice contribute about 90%.[1] (Payyappallimana, 2010). Herbal medicine-based traditional medical system of treatment has continued to grow in relevance in many countries of the world despite the onslaught of modern orthodox medicine.

According to the World Health Organization (WHO), 60% of the world's population relies on herbal medicine, about 80% in developing nations depending almost entirely on it for their primary health care needs while the trade in medicinal plants, herbal raw materials and herbal drugs is growing at annual growth rate of about 15%.[2] (Sajjad, Khan & Ahmad, 2019). In developing, middle and low income countries, the reasons for this is difficulties in accessing orthodox medications due to such factors as affordability, familiarity, as well as the low ratio of doctors in rural areas compared to towns and cities. "The ratio of traditional healers to the population in Africa is 1: 500 compared to 1:40 000 medical doctors" [3](Abdullahi, 2011).In highly rural areas modern medical professionals are greatly outnumbered by traditional healers, sometimes by a ratio as high as 100:1[4],[5] (Merriam & Muhamad, 2013; King, 2000). In addition, traditional healers provide more to their communities by serving as counselors giving spiritual and psychological comfort, and as 'historians' preserving their cultures [6] (Owumi, 2002).

In developed countries increasing awareness of the limitations of modern medicine and rising interest in preventive healthcare habits are some of other factors that have helped the increasing popularity of traditional medicine. As environmental issues continue to build up more natural and more eco-friendly ways of living become the better option as traditional and alternative treatments present "green" approach to healthcare and lifestyle[7] (Coulter & Willis,2004). Phytochemicals from plants are also considered to have more beneficial biological activities (such as antimicrobial, antioxidant, antidiarrheal, anticancer, analgesic and wound healing activity), less resistance, more effective with less adverse effects and more versatility than chemically synthesized drugs[8] (Ingle et.al, 2017).

The initial setbacks of traditional medicine (non-standardization of dosage, use of crude, unsorted formulations, absence of manufacture or expiry dates, ignoring of side effects of adjoining compounds, little or no quality control measures and un-verifiable involvement of

metaphysical secrets and magic all connect to its heavy reliance on trial and error. Since applied or ingested substances may harm or be fatal to bodily systems the new approach to diseases management is based on scientific observation of the dialogue between medicinal formulations and the hosts [9](Lemonnier et.al, 2017).

A recent WHO declaration adopted at the Global Conference on Primary Health Care asserted that "the success of primary health care will be driven by applying scientific as well as traditional knowledge, and extending access to a range of health care services, which include traditional medicines" [10](WHO,2019). A survey of the major challenges to regulation in the practice of traditional and complementary medicine covering 133 nations showed that 99 quoted lack of research data as their top challenge[10] (WHO,2019).

Investigating and understanding traditional medical practices and ethno–medicine have helped empirically to identify plant materials containing constituents with potential applications to modern medicine[11],[12] (Mamedov, 2012; Musheer et.al, 2019). Drugs and pharmaceutical researchers have for long acknowledged the importance of this by hiring traditional medicine practitioners as consultants for their wide knowledge of the plants and substances they use, in order to maximize the efficiency of patient healthcare, especially in developing countries.

Many bogus claims on herbal remedies remain largely unproven. "A shocking 2/3rds of all herbal supplements have never been clinically proven to work and that usually means quite simply that they don't work" [13](Manfredi, 2017). However, in many cases efficacy has been proven by phytochemical profiling, biological evaluation, up to isolation of bioactive phyto-constituents and novel compounds. The root decoction of *Croton menyhartii* Pax (Euphorbiaceae) used as a malaria remedy in East Africa was confirmed by *in vitro* tests in South Africa to have a very high degree of anti-plasmodia activity. Tests in 2001 had earlier revealed similar activity in plant in the same genus, *C. pseudopulchellus* [14](Fowler, 2006).

Indigofera nummulariifolia (Livera) Ex. Alston (Fabaceae) is a medicinal plant found in tropical and subtropical regions of the world such as India, Nigeria, Madagascar, Australia and parts of Asia reportedly used by ethnic communities treat 'liver complaints'[15] (Santhosh et.al, 2019). This use is without scientific investigation although many other species of the Indigofera genus have been proven to have a vast array of pharmacological activities. *I. pulchra* is known to have anti-venom properties[16] (Abubakar et.al, 2006), *I. heterantha* is used as herbal medicine as well as folk medicine to treat gastrointestinal disorder and abdominal pain, *I. tinctoria* has shown antioxidant, free radical scavenging activity, and anti-dyslipidemic activity, and *I.oblongifolia* proven to possess antimicrobial, hepatoprotective and strong lipoxygenase inhibitory activity [17],[18](Halim, Zeb & Khan, 2018 ;Mohammad & Choudhary, 2011). In this study *I.nummulariifolia* (whole plant) was extracted using four (4) solvents: n-hexane, dichloromethane, ethyl acetate and methanol, after which they were screened for phytochemicals, and antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus flavus*.

2. MATERIALS AND METHODS

2.1 Collection and identification of plant materials

I.nummulariifolia was harvested in February, 2018 from different locations in the local forests of Aieje village in Edumoga District, Okpokwu Local Government Area of Benue State, Nigeria. The plant material was identified (and the herbarium samples stored) in the Department of Biological Sciences, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria.

2.2 Preparation and Extraction of Plant Material

About 1kg of harvested plant was shade-dried, pulverized and shared into two. One half was subjected to exhaustive Soxhlet Extraction (SE) with four selective solvents in order of increasing polarity (n-hexane, dichloromethane, ethyl acetate and methanol). The cycle was repeated on the other half but using Microwave-Assisted Extraction (MAE). The extracts for each solvent and method were concentrated with the aid of a vacuum pump and left to dry to constant mass.

2.3 Qualitative phytochemical Screening

The extracts obtained were tested for the presence glycosides, tannins, flavonoids, saponins, cardiac glycosides, anthraquinones, alkaloids, steroids and triterpenoids using the standard procedures[19],[20] (Ciulei,1983; Banu & Cathrine, 2015).

2.4 Culture Media and Microbes

The following growth media were used in the antimicrobial activity screening and were all prepared according to manufacturer's instructions: Nutrient agar (AcoumixTM), Nutrient broth (HKM), Mueller Hinton agar (Titan Biotech), Sabourand Dextrose agar (Titan Biotech), Sabourand Liquid Medium and Normal saline. Activity was checked against the following microorganisms: (bacteria) *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*; (Fungi) *Candida albicans* and *Aspergillus flavus*. They were sourced from the Department of Pharmaceutical Microbiology Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Ciprofloxacin (anti-bacterial) and Terbinafin (anti-fungi) were used respectively as positive antibacterial and antifungal controls.

2.5 Sensitivity Test for Antimicrobial Activity

The Agar Well Diffusion Method[21],[22] (Singleton, 1981; Ghatage, et.al, 2014) was adopted for antimicrobial sensitivity tests. 20ml each of sterile Mueller Hinton Agar prepared following manufacturer's instruction were poured into sterile Petri dishes and allowed to solidify. 2ml of each culture standardized with 0.9% normal saline (McFarland turbidity standard) were flooded over each dish and the

excess discarded. 8mm diameter equidistant wells were bored into the seeded agar using flamed cork borer and labeled. Each well was filled with 100µL of the appropriate extract at 100mg/ml and lower concentrations, and standard drugs at 50µg/mL. The plates were loaded in duplicate and left for one hour to allow for proper diffusion before incubating upright in the dark. After 24 hours (at 37°C) for bacteria and 72 hours (at 30°C) for fungi they were observed for growth and the diameters of any resulting zones of inhibition (ZOI) formed around wells measured in millimeter scale using a transparent rule.

2.6 Minimum Inhibitory Concentration

The Broth Dilution methods were used. The following concentrations (mg/mL): 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, 0.390625 and 0.1953 of plant material were prepared by two-fold serial dilution in Muller Hinton agar (for bacteria) and Sabourand Dextrose agar (for fungi) petri dishes. Sterile micro filter paper discs for each of the six organisms were placed on the solidified agar and 10µl of each standardized culture was then added and left for an hour to diffuse. The loaded dishes were incubated in the dark at 37°C for 24 hours (bacteria) and 30°C for 72 hours (for fungi). They were then observed for visible growth by turbidity. The lowest concentration at which no detectable bacterial or fungal growth occurred was taken as Minimum Inhibitory Concentration (MIC).

2.7 Minimum Bactericidal / Fungicidal Concentration

The minimum bactericidal/fungicidal concentration of the extracts was determined from the tubes that showed no visible growth in the MIC determination. The micro filter paper discs bearing the organisms were lifted and seeded on fresh 5ml sterile Nutrient Broth (bacteria) and Sabourand Liquid medium (fungi) in labeled bottles. The incubation cycles were repeated. The lowest concentration of the extracts that showed no visible colony growth on the medium was regarded as minimum bactericidal/fungicidal concentration.

3. RESULTS AND DISCUSSION

3.1 Comparative Extractive Yield of Extracts

Table-1 shows that Soxhlet Extraction with higher recovery yields provided a better extraction efficiency for *I.nummulariifolia* than Microwave Assisted Extraction. The decreasing order of extractive yields by solvents in both methods is Methanol (most polar), N-hexane (least polar), ethyl acetate and dichloromethane. Extraction is according to polarity. Ethanol extracts essentially polar substances while N-hexane extracts non-polar (fatty) substances. Ethyl acetate and dichloromethane extract moderately polar and moderately non-polar substances respectively. Thus a preponderance of highly polar (high boiling) and non-polar phyto-constituents in the plant is suspected compared to compounds of moderate polarity. However, extractive yields are generally low, with dichloromethane having as low as 0.38% and 0.1% (for SE and MAE respectively). Low yields of crude extracts is in tandem with the usual occurrence of bioactive compounds in amounts that hardly meet their demand for medicinal applications, a factor that has since promoted resort to their synthesis [23] (Beutler, 2009).

Table-1 Comparative Extractive Yield of *Indigofera nummulariifolia*

SOLVENT	PERCENT EXTRACTIVE YIELD (%)	
	SOXHLET EXTRACTION	MICROWAVE-ASSISTED EXTRACTION
N-hexane	0.56	0.27
Dichloromethane	0.38	0.10
Ethyl acetate	0.50	0.18
Methanol	3.46	1.01

3.2 Phytochemical Screening of Extracts

The methanol extract tested positive for most classes (glycosides, tannins, saponins, flavonoids, cardiac glycosides, steroids/triterpenes, alkaloids) except anthraquinones (Table-2). Cardiac glycosides and steroids/triterpenes tested positive in the ethyl acetate extract while the dichloromethane and n-hexane extracts indicate the presence of alkaloids and steroid/triterpenes. *I.nummulariifolia* may contain physiologically active compounds across most classes of natural products especially steroids/triterpenes. With reference to ethno-medicinal utility of the plant to treat 'liver complaints'[15](Santhosh et.al, 2019) the positive phytochemical test for flavonoids in the methanol extract, is also instructive since they, reportedly, exhibit anti-hepatotoxic activity[24] (Agrawal, 2011).

Table-2 Phytochemical Screening of *I.nummulariifolia* Extracts

PHYTOCHEMICALS	TESTS	N-HEXANE EXTRACT	DICHLORO-METHANE EXTRACT	ETHYL ACETATE EXTRACT	METHANOL EXTRACT
Glycosides	a. Fehling's Test	-	-	-	+
	b. Ferric Chloride Test	-	-	-	+
Tannins	a. Ferric Chloride Test	-	-	-	+
	b. Bromine Water Test	-	-	-	+
	c. Lead Sub-acetate Test	-	-	-	+
Flavonoids	a. Shinoda Test	-	-	-	-
	b.Sodium Hydroxide Test	-	-	-	+
	c. Ferric Chloride Test	-	-	-	+
Saponins	a.Frothing Test	-	-	-	+
	b. Haemolysi				

	s Test	-	-	-	+
Cardiac glycosides	a. Keller-Killiani Test	-	-	+	+
	b. Kedde's Test	-	-	+	+
Anthraquinones	a. Free Anthracenes	-	-	-	-
	b.Combined Anthracenes	-	-	-	-
Alkaloids	a. Dragendoff's Test	+	+	-	+
	b. Wagner's Test	+	+	-	+
	c. Meyer's Test	+	+	-	+
Steroids and triterpenoids	a. Salkwoski's Test	+	+	+	+
	b. Liebermann Buchard Test	+	+	+	+

Key: + = positive test result ; - = negative test

3.3 Sensitivity Test (Zones of Inhibition of Extracts)

Table-3 shows zones of inhibition (ZOI) of microbial growth by extracts. All the extracts show some antibacterial activity against the selected strains with ZOI in the range 13mm-23mm. The n-hexane extract has the highest ZOI of 23mm against *Salmonella typhi*. With the exception of methanol extract, all the other extracts also show antifungal activity, with ZOI in the range 16mm-19mm.Dichloromethane extract (with 19mm each) and ethyl acetate extracts (with 18mm and 19mm) have the highest antifungal ZOI's against *Candida albicans* and *Aspergillus flavus* respectively.

EXTRACT	MICROORGANISM	A	B	C	D	E	F	G	H	I	J
N-Hexane	Staphylococcus aureus	-	-	-	+	+	+	+	+	+	+
	Escherichia coli	-	-	-	+	+	+	+	+	+	+
	Salmonella typhi	-	-	-	-	+	+	+	+	+	+
	Bacillus subtilis	-	-	-	+	+	+	+	+	+	+
	Candida albicans	-	-	+	+	+	+	+	+	+	+
	Aspergillus flavus	-	-	+	+	+	+	+	+	+	+
Dichloromethane	Staphylococcus aureus	-	-	-	+	+	+	+	+	+	+
	Escherichia coli	-	-	-	+	+	+	+	+	+	+
	Salmonella typhi	-	-	+	+	+	+	+	+	+	+
	Bacillus subtilis	-	-	+	+	+	+	+	+	+	+
	Candida albicans	-	-	-	+	+	+	+	+	+	+
	Aspergillus flavus	-	-	-	+	+	+	+	+	+	+
Ethyl acetate	Staphylococcus aureus	-	-	+	+	+	+	+	+	+	+
	Escherichia coli	-	-	-	+	+	+	+	+	+	+
	Salmonella typhi	-	-	-	+	+	+	+	+	+	+
	Bacillus subtilis	-	-	+	+	+	+	+	+	+	+
	Candida albicans	-	-	-	+	+	+	+	+	+	+
	Aspergillus flavus	-	-	-	+	+	+	+	+	+	+
	Staphylococcus aureus	-	-	+	+	+	+	+	+	+	+
	Escherichia coli	-	+	+	+	+	+	+	+	+	+
Methanol	Salmonella typhi	-	+	+	+	+	+	+	+	+	+
	Bacillus subtilis	+	+	+	+	+	+	+	+	+	+
	Candida albicans	+	+	+	+	+	+	+	+	+	+
	Aspergillus flavus	+	+	+	+	+	+	+	+	+	+

Table-3 Zones of Inhibition (mm) of Extracts of *I.nummulariifolia* against Microorganisms

EXTRACT	ORGANISM	100(mg/mL)	50 (mg/mL)	25 (mg/mL)	12.5 (mg/mL)	*CIP 50µg/mL	*TBF 50µg/mL
N-hexane	*Staphylococcus aureus*	18	16	12	0	30	
	Escherichia coli	19	17	14	12	22	
	Salmonella typhi	23	20	16	14	20	
	Bacillus subtillis	17	14	0	0	24	
	Candida albicans	17	14	12	0	-	25
	Aspergillus flavus	16	13	0	0	-	33
Dichloromethane	*Staphylococcus aureus*	18	16	13	0	30	
	Escherichia coli	18	16	14	0	22	
	Salmonella typhi	17	14	0	0	20	
	Bacillus subtillis	16	13	0	0	24	
	Candida albicans	19	17	14	0	-	25
	Aspergillus flavus	19	17	14	0	-	33
Ethyl acetate	*Staphylococcus aureus*	15	0	0	0	30	
	Escherichia coli	18	15	12	0	22	
	Salmonella typhi	18	15	13	0	22	
	Bacillus subtillis	15	0	0	0	24	
	Candida albicans	18	15	0	0	-	25
	Aspergillus flavus	19	17	14	0	-	33
Methanol	*Staphylococcus aureus*	16	13	0	0	30	
	Escherichia coli	14	0	0	0	22	
	Salmonella typhi	13	0	0	0	20	
	Bacillus subtillis	13	0	0	0	24	
	Candida albicans	0	0	0	0	-	25
	Aspergillus flavus	0	0	0	0	-	33

*CIP=Ciprofloxacin (anti-bacterial); **TBF = Terbinafin (anti-fungi)

3.4 Minimum Inhibitory Concentration (MIC) of Extracts

From Table-4 (minimum inhibitory concentration (MIC)) the N-hexane extract has the lowest antibacterial MIC of 12.5mg/mL for activity against *Salmonella typhi*. Its MIC for activity against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* is 25mg/mL. The dichloromethane extract has the same MIC (25mg/mL) for *Staphylococcus aureus* and *Escherichia coli* while the ethyl acetate extract has it for *Escherichia coli* and *Salmonella typhi*. The lowest antifungal MIC's of 25mg/mL is observed for the dichloromethane and ethyl acetate extracts against *Candida albicans* and *Aspergillus flavus*.

Table-4 Minimum Inhibitory Concentration of *I.nummulariifolia* Extract

Key: + = Growth, - = No growth

CONCENTRATIONS (mg/mL)									
A	B	C	D	E	F	G	H	I	J
100	50	25	12.5	6.25	3.125	1.5625	0.78125	0.3906	0.1953

3.4 Minimum Bactericidal/Fungicidal Concentration of Extracts

Table-5 (MBC/MFC) shows that the lowest concentration of a plant extract that killed organism is 50mg/mL for activity against Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus subtilis, Candida albicans and Aspergillus flavus. It is 100mg/mL for the other organisms for which the plant extracts had activity except for ethyl acetate extract (against Bacillus subtilis) and methanol extract (against Escherichia coli and Salmonella typhi) where the highest concentration (100mg/mL) that inhibited growth (Table-4) of organisms could not kill them.

Table-5: Minimum Bactericidal/Fungicidal Concentration of *I.nummulariifolia* Extract

EXTRACT	MICROORGANISM	A	B	C	D	E	F	G	H	I	J
N-hexane	*Staphylococcus aureus*	-	-	+	+	+	+	+	+	+	+
	Escherichia coli	-	-	+	+	+	+	+	+	+	+
	Salmonella typhi	-	-	+	+	+	+	+	+	+	+
	Bacillus subtilis	-	+	+	+	+	+	+	+	+	+
	Candida albicans	-	+	+	+	+	+	+	+	+	+
	Aspergillus flavus	-	+	+	+	+	+	+	+	+	+
Dichlorometh ane	*Staphylococcus aureus*	-	-	+	+	+	+	+	+	+	+
	Escherichia coli	-	-	+	+	+	+	+	+	+	+
	Salmonella typhi	-	+	+	+	+	+	+	+	+	+
	Bacillus subtilis	-	+	+	+	+	+	+	+	+	+
	Candida albicans	-	-	+	+	+	+	+	+	+	+
	Aspergillus flavus	-	-	+	+	+	+	+	+	+	+
Ethyl acetate	*Staphylococcus aureus*	-	+	+	+	+	+	+	+	+	+
	Escherichia coli	-	-	+	+	+	+	+	+	+	+
	Salmonella typhi	-	-	+	+	+	+	+	+	+	+
	Bacillus subtilis	+	+	+	+	+	+	+	+	+	+
	Candida albicans	-	-	+	+	+	+	+	+	+	+
	Aspergillus flavus	-	-	+	+	+	+	+	+	+	+
Methanol	*Staphylococcus aureus*	-	+	+	+	+	+	+	+	+	+
	Escherichia coli	+	+	+	+	+	+	+	+	+	+
	Salmonella typhi	+	+	+	+	+	+	+	+	+	+
	Bacillus subtilis	+	+	+	+	+	+	+	+	+	+
	Candida albicans	+	+	+	+	+	+	+	+	+	+
	Aspergillus flavus	+	+	+	+	+	+	+	+	+	+

Key: + = Growth, - = No growth

CONCENTRATIONS (mg/mL)									
A	B	C	D	E	F	G	H	I	J
100	50	25	12.5	6.25	3.125	1.5625	0.78125	0.3906	0.1953

Affirmative antimicrobial results(ZOI,MIC and MBC/MFC) observed for the plant extracts against various microorganisms may have weighty implications. *Salmonella typhi* is gram negative and implicated in the attack of typhoid fever. *Escherichia coli* is gram negative and the causative agent for cystis and food poisoning. *Bacillus subtilis*, a gram positive bacteria, causes meningitis, ear and urinary tract infections. *Staphylococcus aureus* is gram positive and responsible for cellulites, conjunctivitis, food poisoning and pneumonias[25][21] (Okafor & Ovurevu, 2017; Singleton, 1981). On the other hand the fungus *Candida albicans* is one of the causative agents for oral thrush, urinary tract and genital yeast infections while *Aspergillus flavus* is linked to aspergillosis, hepatotoxicity, genotoxicity, carcinogenicity, nephrotoxicity and immunosuppression[26] (Satish, et. al, 2007). Activities against gram positive and gram negative bacterial strains as well as fungal strains by the plant extracts could mean therapeutic potency against diseases attributed to the six selected strains and possibly against more. The crude use of the plant to treat 'liver problems'[15] (Santhosh et.al, 2019), most of which are bacterial in nature, seems justified. A related plant, *I.oblongifolia*, had earlier been reported to possess antimicrobial, hepatoprotective and strong lipoxygenase inhibitory activity[17],[18] (Halim, Zeb & Khan, 2018 ;Mohammad & Choudhary, 2011)

4. CONCLUSIONS AND RECOMMENDATIONS

Positive phytochemical tests for critical phyto-constituents and observed antimicrobial activity of extracts of *Indigofera nummulariifolia* against the selected organisms have lent credence to ethno-medicinal use the plant to treat liver complaints and also strongly suggests broad spectrum potency. Trends from some related plants reveal antimicrobial, hepatoprotective and strong lipoxygenase inhibitory activity, with liver-protection capacity already confirmed[27] (Jannu et.al, 2012). The liver is known to be affected by ailments that affect other organs and tissues of the body[28],[29] (Schnabl and Brenner, 2014; Vajro et.al, 2013), further supporting expected potency of our plant against many more microbes. The plant should be tested further for activity against more gram positive and gram negative bacteria, and fungi. A recent review of the phytochemistry and pharmacology of the Genus Indigofera [30](Rahman et.al, 2018) documented 65 phytochemicals with various bioactivities. *I.nummulariifolia* should also be investigated for useful bioactive compounds.

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