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Gas chromatography/Mass spectroscopy analysis of *Catha edulis* Forsk, A psycho stimulant revealed potent solvent dependent antimicrobial activity

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Abstract
Aim: The present study was aimed to identify the best solvent system for the extraction of bioactive compound of *Catha edulis* Forsk (Also Known as Khat, qat) and to validate the antimicrobial activity of these extract against the clinically isolated resistant bacterial strains.
Background: *Catha edulis* Forsk is by far the most commonly cultivated shrub native to East Africa and Arabian Pennisula. The fresh leaves of this plant are chewed traditionally for feeling of wellness, mental alertness and euphoria by the people in Saudi Arabia. Studies demonstrated the cytotoxic and therapeutic effect of *Catha edulis*, however this study is first of its kind to demonstrate the solvent dependent antimicrobial activity in clinically isolated microbial strains.
Methodology and Results: The extract was prepared in three different polar solvents (Methanol, DMSO and Water) to determine their relative capacity to fish out the phytoconstituents with good antimicrobial, antioxidant and anti-inflammatory activity by performing a comparative phytoconstituent analysis using GC/MS. The antimicrobial activity of these extracts was tested against the clinical isolates such *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebseilla pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa* and *Candida albicans* by agar well diffusion assay. For all the isolates, identification and antibiotic susceptibility was determined by Vitek-2 system. The results showed that out of three polar solvents, extract prepared in DMSO and methanol showed significant antimicrobial activity. Notably, the extract in DMSO was active against the multi-drug resistant isolates. This differential antimicrobial activity is concurrent with phytoconstituents detected by GC/MS.
Conclusion: The study unveils *Catha edulis* Forsk as a potential source of natural antimicrobial compound against both sensitive and MDR bacteria. Moreover, this is the first report to demonstrate the effect of solvent on the differential extraction of various phytoconstituents of *Catha edulis* by GC/MS.
Significance and impact of the study: Study distinct itself by corroborating observed differential antibacterial activity with the phytoconstituent detected by GC/MS analysis. Also, this is the first report to demonstrate the effect of solvent on differential extraction of phytoconstituent showing strong antimicrobial, anti-inflammatory and antioxidant activity of *Catha edulis* Forsk.

Keywords: *Catha edulis*/Khat; multidrug resistance bacteria antimicrobial activity; Gas Chromatography/Mass spectroscopy

Introduction
In the last few decades healthcare system has made considerable improvement; discovery of antibiotics for example has greatly revolutionized the quality of healthcare provided for infectious diseases to the extent that they have been termed as "Wonder drugs". It is needles to reiterate how significantly it has contributed globally to control the infectious diseases that are still the leading cause of morbidity and mortality, especially in the developing countries. Regrettably extensive usage put selective pressure on microbes and in retaliation they evolve and exploit multiple mechanisms (genetic, physiological and biochemical) of resistance to each and every synthetic antibiotic introduced into the clinical practice. This eventually led to the relentless development of multi drug resistance bacteria's (Powers 2004) [33]; resistant to a range of synthetic antibiotics. Therefore addressing the emerging problem of antimicrobial resistance has become a challenging requirement today. Owing to the fact that antibiotic resistance is inevitable, the search for novel antibiotics is a natural response. It also explains the interest towards evaluating plant derived biologically active compounds as a possible antimicrobial chemotherapy agent (Pretorius, Magama *et al*. 2003 [34], Moreillon, Que *et al*. 2005 [26], Niño, Mosquera *et al*. 2012 [30], Djeussi, Noumedem *et al*. 2013) [10]; mostly because of their low toxicity.

Plants produced wide variety of secondary metabolites such as tannin terpenoids, alkaloids, flavonoids, glycosides etc with relatively strong antimicrobial activity invitro (Cowan 1999 [7], Dahanukar, Kulkarni *et al*. 2000) [8]. There are many studies on use of plant extracts and their phytochemicals as potent antimicrobial agent (Scalbert 1991 [37], Janovska, Kubikova *et al*. 2003 [16], Burt 2004 [5], Mahesh and Satish 2008 [23], Joshi, Lekhak *et al*. 2009 [17], Rajendran and Ramakrishnan 2009) [35] and most of them succeeded in demonstrating bactericidal activity of plant extracts on resistant strains (Nascimento, Locatelli *et al*. 2000 [27], Khan, Islam *et al*. 2009) [20]. However, their efficacy in therapeutic regime needs rigorous evaluation.

In an effort to expand the spectrum of novel antimicrobial molecule from natural herbal resources we selected *Catha edulis* Forsk (also known as khat, qat) which is an evergreen flowering plant of family Celestraceae. The plant is commonly cultivated in Eastern African and South- West Arabian Pennisula. Normally, The leaves and twigs of this plant is chewed by Yamanites, Africans and Saudis especially in the south western region (Jazan) where its prevalence is reported to be 21.1% (Ageely 2009) [1] for its natural psycho stimulatory effect (Luqman and Danowski 1976 [22], Kalix 1996 [19], Kite, Ismail *et al*. 2003) [21]. It contains an alkaloid called cathinone which is responsible for psychotic stimulating effects however, wide variety of other polyphenolic compounds such as tannins, triterpenes, flavonoid including small amounts of essential oils, sterols, thiamine, riboflavin, niacin, iron and amino acids are reported (Organization 2006) [31]. The potential of these compounds as an antimicrobial agent has been selectively reported (Elhag, Mossa *et al*. 1999 [11], Al-hebshi 2006 [3], Al-haroni *et al*. 2006, Naveed Ahmed 2012) [28] but the details of active phyto constituent are not studied so far. Moreover mostly the studies focus on normal strains for example El hag *et al* reported tingenone and 22 β hydroxytingenone as an active phyto constituent responsible for antimicrobial activity. However aforementioned study was carried out in callus culture rather than in fresh plant [Elhag H 1999] [11].

This study is of its first kind to report the antimicrobial activity of *Catha edulis* Forsk on clinical isolates that are resistant towards various antibiotics. It also involves determining a better solvent for the preparation of *Catha edulis* extract. Moreover the photochemical components of extracts in different solvents were determined using GC /MS and there corresponding antimicrobial activity was corroborated.

Materials and methods

Plant Material: Bundles of *Catha edulis* Forsk shoots and small branches of about 2000 g (the same type that chewers purchase and consume locally) were obtained fresh from Substance Abuse Research Centre, Jazan University. The plant was identified by a taxonomist of Substance Abuse Research Centre and a voucher specimen is available in the department for reference under a voucher number 001

Preparation of crude extract from *Catha edulis* Forsk*:* Relatively fresh materials of *Catha edulis* Forsk were washed with distilled water to remove dust and debris and then subjected to air-drying in a dark place. After 2 weeks, the dried materials weighing 1.0, 2.5, 5.0 and 10 gms was soaked in 100 ml of water, DMSO and methanol respectively in a water bath at 25°C for 24 h with continuous shaking using Cole Palmer Orbital Shaker at 40 g. Subsequently, leaves were homogenized and the extract was filtered using whattman No1filter paper and concentrated at 40°C under reduced pressure (Riaz, Faisal *et al*. 2011). The crude extract was finally diluted to obtain 100 µg, 50 µg, 25 µg, 12.5 µg in 0.1ml of respective solvents.

Bacterial isolates: Bacterial strains included in the study were isolated from outpatient attending the clinics in King Fahd Central Hospital, Jazan, Saudi Arabia. The bacterial cultures were obtained from the specimens like urine, throat swab, wound swab and skin scrapings etc. The samples were cultured on selective media's like urine samples were cultured on Macckoney agar, CLED agar while throat and wound swabs samples were inoculated on Blood agar, Manitol Salt agar and Macckoney agar. Additionally, the samples collected from skin scrapping were cultured on Sabarauds agar and incubated at 28º C for 48 hours. Following incubation the colonies grown were purified by repeated sub culturing using quadrant plate technique. The pure colonies were first identified by conventional method based on Gram's staining, colony morphology and biochemical test. For Biochemical analysis and for the determination of MIC Vitek 2 system was used. Currently, the species which more frequently causes infection in humans were selected for the study. Informed written consent was obtained prior to the enrolment of subject in the study according to the institutional ethical guidelines. Study was approved by ethical committee of Substance Abuse Research Centre, Jazan University.

Identification of clinical isolates with VITEK 2 system: Further strain identification and antibiotic susceptibility test was performed using new VITEK 2 compact ID/AST systems (bioMérieux Marcy l'Etoile, France) which uses colorimetric reading for identification of gram-negative and gram- positive bacteria according to manufacturer's instructions (Pincus 2006). Bacterial culture suspension was made in 0.45% sodium chloride equivalent of 0.5 McFarland turbidity standards. Both GP and GN card were automatically filled by a vacuum device, sealed and inserted into the vitek-2 reader incubator module at temperature of 37ºC. The results were expressed with the confidence value as excellent identification (96-99%), very good identification (93-95%), good identification (89-92%), acceptable identification (85-88%), low discrimination (result required few additional test such as oxidase, bacterial motility, indole, hemolysis or pigmentation to confirm the correct identification) and no identification. Correct identification was defined as the association of excellent identification and low discrimination. Results were expressed in numbers and percentages.

Determination of antibiotic susceptibility test for clinical isolates using Vitek-2 system: All the clinical isolates that had excellent identification were further evaluated for antibiotic susceptibility testing using Vitek 2 compact ID/AST system according to the instructions of manufacturer (bioMérieux Marcy l'Etoile, France). Briefly, bacterial suspension was prepared in 0.45% sterile normal saline to a density equal to 0.5 McFarland turbidity standards (NCCLS 2000). AST cards (AST N-292, AST P-580) were automatically filled sealed and loaded into carousel incubator for 15- 16 hours. The data were collected at every 15 minutes interval during the incubation period. The details of drugs contained in the AST cards are mention in the Table 1 and 2.

Determination of antibacterial activity of crude *Catha edulis* Forsk extract: The antimicrobial activity of crude

Catha edulis extract was tested using well in agar diffusion assay (Ncube, Afolayan *et al*. 2008, Das, Tiwari *et al*. 2010). The bacterial cultures were grown in nutrient agar medium (Oxoid, UK) at 37 °C. After 24 h of growth, 100 µl of inoculum at a concentration of 10^8 CFU/mL (0.5% McFarland) was spread on Muller-Hinton agar media (Oxoid, UK) to ensure uniform lawn growth (Ferraro 2001, Wikler 2006, Mathew A 2007). The agar plate was divided into quadrant and label accordingly to minimize the error. 8mm of hole was punched with sterile cork borer aseptically in middle of the plate and the hole was filled with 100 µl of *Catha edulis* extract prepared in three different solvents. The plates were incubated at 37°C for 24hrs and the zone of inhibition was measured to determine its possible antimicrobial activity against the clinical isolates included in the study. For positive control 100 µg of Gentamicin was used and 100µl of solvents (water, methanol and DMSO) alone was used as internal negative control. The final concentration of DMSO and methanol used was less than 2.5%, and thus did not affect the microbial growth. The breakpoint for zone of inhibition was measured according to EUCAST guidelines. The break point around Gentamycin for *Enterobacteriacea* members is 14mm, for *Staphylococcus aureus* is 18mm, while for *Pseudomonas aeruginosa* is 15mm and for *Streptococcus* group A,B,C and G its 18mm (Erythromycin) (Kahlmeter, Brown *et al*. 2006). All the experiments were carried out in triplicate to minimize the experimental bias on at least three separate occasions.

Gas Chromatography Mass Spectroscopy (GC MS) Analysis of *Catha edulis* Forsk extract: Presence of various bioactive compounds in the three different extracts of *Catha edulis* was identified by GC MS (Model; QP 2010 Plus, Shimadzu,Tokyo, Japan) outfitted with aVF-5ms fused silica capillary column of 30mm length, 0.25mm ID, and 0.25 μm df. Ionization of sample components was performed in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 0.96 ml /min and an injection volume of 2.0 µI. The injector temperature was 250 ºC; ion-source temperature 280 ºC. Mass spectra were taken at a scan interval of 0.5 seconds and fragments from 40 m/z – 450 m/z. Total GC running time is 36 min and the compounds were identified based on their retention time, retention indices, and mass spectra. The name, molecular weight and structure of the components of the test materials were ascertained [7] comparing the spectrum of the unknown compound with the spectrum of the known compound stored in the database of National Institute of Standard and Technology (NIST) library. The relative percentage amount of each compound was calculated by comparing its average peak area to the total areas. Software version 2.71 was used to analyze mass spectra chromatograms.

Results

Identification of clinical isolates: After the identification of clinical isolates by conventional method, all the strains were further accurately identified up to species level using Vitek-2 ID/AST compact system. The strains identified were *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Candida albicans* with confidence value of 96-99% (excellent identification).

Susceptibility test using VITEK 2 system: The antibiotic susceptibility and resistance patterns for clinical isolates identified using VITEK 2 system revealed varying degree of resistant to antibiotics. Among the clinical isolates *Staphylococcus aureus* was resistant to streptogramins A and B, *Escherichia coli* were resistant to amino glycosides whereas *Pseudomonas aeruginosa* was resistant to cephalosporins, tetracyclines, nitrofurantoin and trimethoprims thus indicating high level of resistance (Table1, 2 and 3).

Evaluating the antimicrobial potential of *Catha edulis* Forsk extract: The antimicrobial activities of *Catha edulis* extract obtained from different solvents against the selected clinical isolates are given in the Table-4. Briefly, the zone of inhibitions measured against the methanolic extract were 17mm for *Staphylococcus aureus*, 29mm for *Streptococcus pyogenes*, 22mm for *Escherichia coli*, 16mm for *klebsiella pneumonia*, 31mm for *Proteus mirabilis, Pseudomonas aeruginosa* 19mm and 28mm for *Candida albicans* respectively.

Added to this, the extract prepared in DMSO displayed comparatively better activity than the methanolic extract. The zone of inhibition for *Staphylococcus aureus* were around 22mm, *Streptococcus pyogenes* 24mm, *Escherichia. coli* 32mm, *klebsiella pneumoniae* 27mm, *Pseudomonas aeruginosa* 22mm. Moreover, *Proteus mirabilis* and *Candida albicans* responded with a greater zone of inhibition (33mm) as compared to other strains (Table 4).

The aqueous extract of *Catha edulis* (100µg) was observed active only on gram positive bacteria, while all the other species showed no zone of inhibition around the well.

Phytochemical analysis of *Catha edulis* Forsk extract by GC MS: Given to the differential antimicrobial activity of three different solvent extracts of *Catha edulis*, we performed a comparative phytochemical evaluation of these extracts by GC MS. Our GC MS analyses bear out a clear effect of solvent on the extraction of phytocompound showing antimicrobial, anti-inflammatory, antioxidant, anti-cancer activity, Antiasthma,

Antiarthritic, Hepatoprotective, Antispasmodic, anti-diuretic, androgenic, Endogenous Sleep inducing lipid etc (Table 5). Among the three solvent, extract prepared in DMSO and methanol revealed 7 compounds with strong antimicrobial and antifungal activity viz Neophytadiene, Phytol, Heptacosanol, Hexacosanol (CAS), Stigmast, Dodecanol, Methyl commate B. Whereas the major phytocompounds with reported antimicrobial activity identified in the aqueous extract are Hexadecane, Nonacosanol, Dodecanol (Figure-1). Although numerous other compounds are also present in *Catha edulis* but would not be detected by GC-MS (or would be thermolabile). Details of phytochemical compounds and their biological activity are listed in the Table 5.

Discussion

The growing problem of hard to treat multidrug resistance bacteria (superbugs), instigate a sense of urgency to search for the newer antimicrobial compound with improved efficacy. In fact another driving factor is the observation that the effectual lifespan of synthetic antimicrobial compound is restricted. Therefore, there is a serious need to search and develop non-synthetic, natural antimicrobial agents. Researchers worldwide are investigating multitude of the bioactive compounds from different parts of plants as an alternative form of treatment against various infections that are difficult to treat by mainstream medicine. For this reason we tested the antibacterial activity of *Catha edulis* extracts prepared in

different solvents against the selected clinical isolates resistant towards various antibiotics. *Catha edulis* is evergreen, easily grown, and naturally occurring herb not requiring special interventions for growth. Normally 100 to 200 g of tender leaves and stems are chewed and the juice is swallowed. Traditionally the leaves and twigs of this plant are also used as a home remedy for cough and influenza (Feyissa and Kelly 2008) chest (Al-Hebshi *et al*. 2010) [2] and stomach problems (Amare and Krikorian 1973).

The results presented above demonstrate that *Catha edulis* could be used as a potential source of effective, natural and non-synthetic antibiotic molecules. Methanolic extract of *Catha edulis* (100 µg) was shown to be effective on all the isolates especially on *Streptococcus pyogenes* which was found to be resistant to virtually all the antibiotics tested, with a zone of inhibition of 29mm. Similarly concentration of 100µg of *Catha edulis* extract in DMSO showed significantly better antimicrobial activity ranging from 22 to 33mm (zone of inhibition) against all isolates except *Streptococcus pyogenes*. It is important to note that the diameters of zone of inhibitions around all the bacterial isolates are above the breakpoints recommended by EUCAST. Equally important outcome is the observation that antimicrobial resistant strains of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeroginosa* were found susceptible to extract prepared in methanol and DMSO. In due course we also detected antimicrobial activity against *Candida albicans* and *Escherichia. coli* which is contradicting the earlier studies (Elhag, Mossa *et al*. 1999 [11], Naveed Ahmed 2012) [28] reporting its activity only against Gram positive bacteria (Elhag, Mossa *et al*. 1999 [11], Al-hebshi 2006 [3], Al-haroni *et al*. 2006 [33], Naveed Ahmed 2012) [28]. Also, our study showed that DMSO and methanolic extract of *Catha edulis* was active on Gram negative bacteria as well. To corroborate these findings and to get an insight into the phytochemical constituents, extracts were analyzed by GC/MS which suggest that the observed results could be due to the presence of strong antimicrobial compounds such as Phytol (diterpine alcohol), Neophytadiene (tripenoid), Stigmast (steroidal) etc (Carretero, López-Pérez *et al*. 2008 [6], Mendiola, Santoyo *et al*. 2008) [25] present in the methanol and DMSO extract only. These bioactive components are well known to exert their antimicrobial activity through different mechanism. Terpenoids causes dissolution of bacterial cell wall (Hernández, Tereschuk *et al*. 2000) [15]. Tannins are known to inhibit the cell wall synthesis by forming irreversible complexes with prolene rich protein (Zablotowicz, Hoagland *et al*. 1996) [39]. The saponins cause leakage of certain proteins and enzymes from the cell wall (Cowan 1999) [7]. Furthermore, steroids are known for their antibacterial activity specifically associated with membrane lipids and cause leakage from liposomes (Epand, Savage *et al*. 2007) [12].

In the case of aqueous extract the maximum antibacterial activity was seen against *Staphylococcus aureus* with the zone of inhibition of 23mm. the observed result could be due to long chain fatty alcohol, dodecanol, detected in the aqueous extract. Previously reported potential antimicrobial activity of dodecanol against the *Staphylococcus aureus*. However, no activity was detected against the microorganism selected for the study suggesting that the aqueous extract either do not hold any phytocompounds with profound antibacterial activity or their threshold level is too less to be effective. Interestingly, the GC/MS analysis of these extracts corroborates with finding of the study in terms of antimicrobial activity. For example, the extracts prepared in methanol and DMSO demonstrate good antimicrobial activity (shows 7 phytoconstituent with strong antimicrobial activity) which is validated with their respective gas chromatogram indicating that *Catha edulis* can be a good source for the formulation of novel antimicrobial compound to combat the prevailing antibiotic resistance. Moreover the results noted above indicate that the antibiotic activity we observed has a broad spectrum ranging from gram positive to gram negative organism under very low concentration thereby minimizing the possible toxic effects. It would be interesting to expand this study in future wherein the inhibitory mechanism of phyto compound with known antimicrobial activity can be unravelled, especially in multidrug resistant bacteria. Our GC/MS analysis also highlights that varying the type of solvent does have differential outcome in terms of extraction of various phytoconstituents with good antioxidant, anticancer, antipyretic, antispasmodic and anti-inflammatory activity (Table 5).

Table 1: Antibiotic susceptibility patterns for Gram positive isolates by Vitek 2 systems.

S. No	Antibiotics	*S. aureus*	*S. pyogenes*
1.	Benzyl penicillin	R	S
2.	Oxacillin/amoxicillin	S	S
3.	Gentamycin	S	S
4.	Tobramycin	S	S
5.	Levofloxacin	S	S
6.	Moxifloxacin	S	S
7.	Erythromycin	S	S
8.	Clindamycin	S	S
9.	Linezolid	S	S
10.	Teicoplanin	S	S
11.	Vancomycin	S	S
12.	Tetracycline	S	S
13.	Tegecycline	S	S
14.	Fosfomycin	S	S
15.	Nitrofurantoin	S	-
16.	Fusidic acid	S	S
17.	Mupirocin	S	-
18.	Rifampicin	I	S
19.	Sulphamethoxazole	S	-
20	Ciprofloxacin	S	S
21.	Cotrimoxazole	S	S

22.	Imepenem	S	R

Table 2: Antibiotic susceptibility patterns for Gram negative isolates by Vitek 2 systems.

S. No	Name of Antibiotics	*E. coli*	*K. pneumonia*	*P. mirabilis*	*P. aeruginosa*
1.	Ampicillin	R	R	S	R
2.	Amoxicillin/Clavulanic Acid	I	S	S	R
3.	Piperacillin / Sulbactam	R	S	S	I
4.	Piperacillin / Tazobactam	R	S	S	I
5.	Cefalotin	R	R	S	R
6.	Cefuroxime	R	R	S	R
7.	Cefuroxime Axetil	R	R	S	R
8.	Cefoxitin	R	R	S	R
9.	Cefpodoxime	R	S	S	R
10.	Cefotaxime	R	I	S	R
11.	Ceftazidime	R	R	S	S
12.	Imipenem	S	S	-	S
13.	Meropenem	S	S	S	S
14.	Amikacin	I	S	S	S
15.	Gentamicin	R	S	S	S
16.	Tobramycin	R	S	S	S
17.	Ciprofloxacin	R	S	S	S
18.	Norfloxacin	R	S	S	S
19.	Tetracycline	R	S	S	R
20.	Nitrofurantoin	S	I	S	R
21.	Trimethoprim/ Sulfamethoxazole	R	S	S	R

Table3: Antibiotic susceptibility patterns for *Candida albicans* by Vitek 2 systems.

S. No	Name of Antibiotics	Interpretation
1.	Flucytosine	S
2.	Fluconazole	S
3.	Voriconazole	S
4.	Amphotericin B	S

Table4: Antimicrobial activity of crude khat extract on clinical isolates.

S. No	Name of Bacteria	Aqueous Extract (µg/0.1ml)				Methanolic Extract (µg/0.1ml)				DMSO Extract (µg/0.1ml)			
		100	50	25	12.5	100	50	25	12.5	100	50	25	12.5
1.	*S. aureus*	23	10	N	N	17	8	N	N	22	14	11	N
2.	*S. pyogenes*	N	N	N	N	29	13	5	N	24	13	7	N
3.	*E. coli*	N	N	N	N	22	10	4	N	32	16	7	4
4.	*K. pneumoniae*	N	N	N	N	16	7	4	N	27	10	4	N
5.	*P. mirabilis*	N	N	N	N	31	15	6	N	33	15	13	N
6.	*P. aeruginosa*	N	N	N	N	19	10	4	N	22	6	N	N
7.	*C. albicans*	N	N	N	N	28	15	4	N	33	13	10	4

Table 5: Details of Phyto compounds detected by GC/MS analysis of *Catha edulis* Forsk and their biological activity

S. No	Compounds Identified	Methanol Extract	DMSO Extract	Water Extract	Chemical Formula	Biological activity **
1.	Cathinone	+	-	+	$C_9H_{11}NO$	Psychostimulatory effect
2	Cathine	+	+	+	$C_9H_{13}NO$	Psychostimulatory effect
3	Neophytadiene	+	+	-	$C_{20}H_{38}$	antipyretic, analgesic, anti-inflammatory, antimicrobial, antioxidant
4	Phytol	+	+	-	$C_{20}H_{40}$	Antimicrobial, anticancer, anti-inflammatory, anti-diuretic
5	Tricosene	+	-	+	$C_{23}H_{46}$	Sex pheromone
6	Nonadecyl pentaflouro propionate	+	-	-	$C_{22}H_{39}F_5O_2$	Surfactant to the functionalization of carbon nanotubes.
7	Heptacosanol	+	+	+	$C_{27}H_{56}O$	Nematicidal, anticancer, antioxidant
8	Hexacosanol (CAS)	+	-	-	$C_{26}H_{54}O$	Antimicrobial and anti-bacterial
9.	Stigmast 5-en 3-ol	+	+	-	$C_{29}H_{48}O$	Antimicrobial Antiasthma, Anti-arthritic, Anti-proliferative and Anti-diabetic
10.	Dodecanol	-	+	+	$C_{12}H_{26}O$	Quorom sensing *C.albicans* antifungal, anti-bacterial
11.	Methyl Commate B	-	+	-	$C_{31}H_{50}O_3$	Antimicrobial, anti-inflammatory
12.	Nonacosanol	-	-	+	$C_{29}H_{60}O$	Antibacterial
13.	Nona decyl triflouroacetate	+	-	-	$C_{21}H_{39}F_3O_2$	Androgenic and anti-spasmodic activity

14.	Octadecenamide	-	+	-	$C_{18}H_{35}NO$	Endogenous Sleep inducing lipid
15.	Hexadecane	-	-		$C_{16}H_{34}$	Antibacterial and antioxidant
16	Heptasiloxane	-	-	+	$C_{16}H_{48}O_6Si_7$	Anti-inflammatory antioxidant
17	Octadecanol	-	-	+	$C_{18}H_{38}O$	Anti-inflammatory antioxidant
18	2, 7 Dimethyl Nonane	-	-	+	$C_{11}H_{24}$	
19	Vitamin E	+	+	-	$C_{29}H_{50}O_2$	Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Antispasmodic, Anti-aging
20	Acetic acid chloro octadecyl ester	+	+	-	$C_{20}H_{39}ClO_2$	
21	1-Nonadecene	+	+	-	$C_{19}H_{38}$	Antibacterial and antioxidant
22	Squalene	-	+	-	$C_{30}H_{50}$	Anti-oxidant free radical scavenger

** **Source:** Dr. Duke's: Phytochemical and Ethnobotanical Databases.

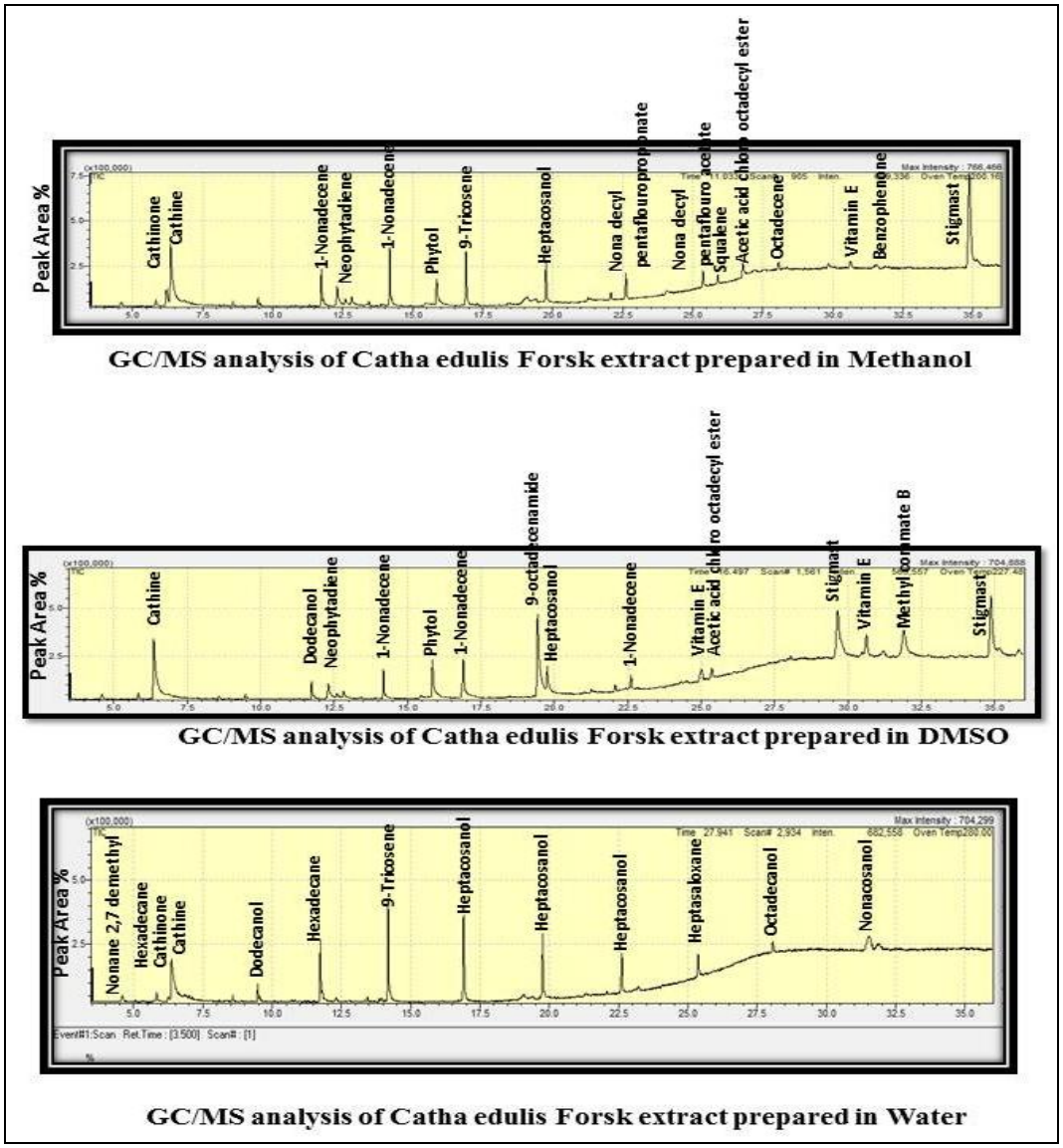

Fig 1: Comparative GC/MS analysis of *Catha edulis* extract prepared in three different polar solvents. Phytocompounds reported as antimicrobial are indicated in the figure.

Conclusion and Future perspective: This study was aimed at identifying the best solvent system to obtained maximum number of phytochemicals especially the phyto compounds with strong anti-microbial activity. Given to all the disadvantages associated with *Catha edulis* abuse (social, economical), we observed that *Catha edulis* do possess many compound with good antimicrobial, anti-inflammatory and antioxidant activity. A further studies involving purification of these phytocompounds individually and determining their mechanistic actions will be the future scope of this study which will help cater their safer application in therapeutics.

Conflict of Interest: All authors have declared that there is no conflict of interest.

Author's Contribution: NF and MR prepared the *Catha edulis* extract and performed antimicrobial assay. BVK performed strain identification and antibiotic sensitivity assay using Vitek system. ASM, MEO extended support for GC/MS, YHH and MYA provided clinical sample and help in drafting the manuscript, SIA and RS provided *Catha edulis* Forsk, AA planned, interprets the results, wrote the manuscript and supervised over all study. All the authors read and approved the final manuscript.

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