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Archana Ashokrao Naik Department of Botany, Savitribai Phule Pune University, Pune, M.S. India

Sabale Suresh Ganpat

Department of Botany, Loknete Vyankatrao Hiray Arts, Science and Commerce College, Panchavati, Nashik, M.S. India

Kale Balasaheb Shantilal

Department of Botany, S.V.K.T. Arts, Science and Commerce College, Deolali Camp, Nashik, M.S. India

Corresponding Author: Kale Balasaheb Shantilal Department of Botany, S.V.K.T. Arts, Science and Commerce College, Deolali Camp, Nashik, M.S. India

In vitro antimicrobial activity of *Fagonia schweinfurthii* Hadidi from Northern Western Ghats, India

Archana Ashokrao Naik, Sabale Suresh Ganpat and Kale Balasaheb Shantilal

Abstract

To assess the phytochemical analysis and antimicrobial activities of *F. schweinfurthii* plant were examined against five different gram positive and gram negative bacteria. Traditionally, *Fagonia* has been used to cure diseases such as skin eruptions, heal sores, skin diseases, anti-pyretic, pain relief, ear infection and venereal diseases. The distilled water, methanol, benzene, chloroform, pet ether & toluene extracts of *F. schweinfurthii* investigated individually for antimicrobial activity (antibacterial activity) by agar well diffusion method. These were investigated against selected bacterial species strains of *Escherichia coli, Klebsiella pneumonia, Bacillus subtilis, Staphylococcus aureus* and *Proteus vulgaris* to find the inhibitory activities of the microbes. The methanol extract of *F. schweinfurthii* stem & root showed considerably high activity against *K. pneumonia* and *E. coli* than other extracts.

Keywords: F. schweinfurthii, medicinal plant, phytochemistry, antimicrobial zone of inhibition

Introduction

Human ancient history says that humans use a plant for a medicinal purpose. Beginning of human civilization, people have been used whole plant or their particular parts like leaves, stems, fruit, flower, seed, and root used as medicine (Mahmood et al., 2005)^[15]. Several plant species have been identified and being used as medicine since ancient history. The major medicinal systems, such as Ayurveda, Siddha, Unani, and Folk (tribal) are being used in India as well as in World (Khare, 2007; Kunwar & Bussmann, 2008) ^[10, 13]. Around 500 plants, their medicinal uses are recorded in ancient texts, and around 800 medicinal plants used in indigenous systems of medicine (Nayanabhirama, 2016)^[18]. Various tribes use these plants for ethnomedicinal purposes (Kumar et al., 2013)^[12]. Plants have been used as medicine, food, etc. since ancient times. Tribal people developed their own traditional knowledge using medicinal plants; these are the national treasure and cultural heritage of our nation. In India about 54 million indigenous tribal people of ethnic communities. These indigenous people have won traditional knowledge about herbal medicines and folk medicines to cure various diseases (Khyade et al., 2011; Sony et al., 2017)^[11, 26]. In the recent era, medicinal plants more focused because they are useful for society to cure various diseases. In medicinal plants presence of secondary metabolites such as alkaloids, glycosides, volatile oil, saponin, tannin, etc. These valuable secondary metabolites used to therapeutics activities such as antibacterial, antifungal, and antioxidant (Najafi & Deokule, 2010; Doctor & Manuel, 2014; Ganorkar & Malpe, 2019) ^[17, 2, 4]. In the world, 35000 to 70000 estimated plant species are used for medicinal purposes (Hashim et al., 2014)^[5].

The Western Ghats (including Sri Lanka) is one of the biodiversity hotspots in India. The Northern Western Ghats hotspot is also known as the 'Sahyadri range' (Myers *et al.*, 2000) ^[16]. The region of Western Ghats consist a rich medicinal recourse, and these medicinal plant sources will be used for pharmacognostic and bioprospecting study. Medicinal flora of Western Ghats is quite rich and its carry more than 62.8% are endemic and medicinally significant. Due to its unique biodiversity, it is one of the important areas with very high value considering bioprospecting of the plant (Rao, 2002) ^[20]. The Western Ghats distribute unique 700 medicinal plants, they are used in traditional and folk medicinal practices (Katole *et al.*, 2018) ^[8]. In the Western Ghats, Selected ethnomedicinal plant use tribal people as deferent therapeutic propose. By using the hidden, unexplored, valuable knowledge of the tribal people for new drug discovery (Kumar *et al.*, 2013) ^[12]. *Fagonia* belongs to the family Zygophyllaceae having 25 Genera's and about 285 species, which are distributed in mainly deserts and dry arid regions of the world (El-Aal *et al.*, 2019) ^[3]. Traditionally, *Fagonia* has been used to cure a diseases such as skin eruptions, in heal sores, skin diseases, anti-pyretic, in

pain relief, ear infection and venereal diseases (Rathore *et al.*, 2011)^[21]. Select these plant species because only, these are ethnomedicinal use in different Indian medicinal systems, and these plant species relatives' plants species also have potentially valuable compounds. These relative plant species have an ethnomedicinal value (Khare, 2007)^[10]. From some selected traditional medicinal plant species isolation and identification of the bioactive compounds and can be used to formulate new drugs to treat various diseases and disorders (Palanisamy & Natesan, 2012)^[19].

Plants extract to use to check antimicrobial resistance mechanism against different bacterial microorganisms using standard antibiotics bark and leaf crud and purified plant extract used as searching for novel principle compounds with biological activities of pharmacological activities like In vitro antibacterial of medicinal plant F. schweinfurthii (Magaldi et al., 2004; Valgas et al., 2007; Jorgensen & Ferraro, 2009; Rathore *et al.*, 2011) ^[14, 28, 7, 21]. The major bioactive chemical constituents of medicinal plants are tannins, alkaloids, flavonoids, terpenoids, phenolics, etc., it has several biological activities (Palanisamy & Natesan, 2012) [19]. Phytochemical compounds are naturally present in medicinal plants or parts such as leaves, fruits, flowers, stems, and roots. Those secondary metabolites have defense mechanisms against pathogenic microorganisms like fungi, viruses, and bacteria. The alkaloid is used in medicines as anesthetic agents (Wadood et al., 2013) [29]. Plant-based isolated bioactive chemical constituents are multifunctional that means isolated bioactive compounds can be used treatment of different diseases (Chithra & et al., 2016). Potential phytochemical leads to searching for new drugs, contributions in pharmacognosy, pharmaceutical, and healthcare products. Secondary metabolites are used for checking biological activities such as antimicrobial activity (Vaghasia et al., 2011; Saxena et al., 2013) [27].

Materials and Method

A) Collection of plant material

Taxonomy & Morphology: Identification and classification of plants using different Floras (Singh *et al.* 2000; Singh *et al.* 2001). Whole plant of *Fagonia schweinfurthii* collected from kesandphata Pune. The plant specimen were identified and authentified by depositing voucher specimen at Botanical survey of India, Regional Office, Western Circle, Pune: 411 001.Maharashtra (India). Above plants parts like Stem and roots collected and dried in shade place. This dried sample make fine powder and used for future analysis.

Extract preparation

Solvent extraction: Fresh plant material collect and this plant material dried under shade condition and successively extracted by Soxhlet's apparatus with different solvents distilled water, Methanol, Benzene, Chloroform, Pet ether, Toluene and Standard Gentamicin. for phytochemical tests and antibacterial activity (Khandelwal & Sethi, 2019)^[9].

B) Phytochemical evaluation

Usually medicinal plant contains active constituents like alkaloids, carbohydrates, flavonoids, anthocyanins, tannins, glycosides, phenols, saponins, starch, lignins, etc. to test their presence different phytochemical tests.

Bacterial culture

Cultures of bacteria were procured from National Collection of Industrial Microorganisms (NCIM), National Chemical

Laboratory, Pune, Maharashtra, India, as shown below. All the test organisms were maintained on nutrient agar slopes and were sub-cultured once in every three-week. These bacteria served as test pathogens for antibacterial activity assay. The known antibiotic Gentamicinis used as a positive control and Dimethyl Sulfoxide (DMSO) used as a negative control in antibacterial assay (Fig. 1).

Test microorganisms

The bacterial cultures used in the study were three are Gram +ve strain Klebsiella *pneumoniae* (NCIM 5082), *Staphylococcus aureus* (NCIM 5022), *Escherichia coli* and (NCIM 2685). Gram -ve strain *Proteus vulgaris* (NCIM 1254) and *Bacillus subtilis* (NCIM 2196).

C) Antibacterial activity

Agar well diffusion method used during study. 20-25 ml. of Nutrient Agar was poured on glass petriplates and allowed to solidify. 20 μ l of reference bacterial strain was spread on respective plate by using spreader, the plate was kept for 30 min. Agar plate punched with a sterile cork bores of 6 mm. size and the sample at a different concentration 25 μ l, 50 μ l and 100 μ l was inoculated. Plates were incubated at 37°C for 24 hours and zone of inhibition was measured. These experiments was performed in triplicates (Sen & Batra, 2012; (Jahangirian *et al.*, 2013) ^[23, 6]. Antimicrobial activity assay using crude extract (Balouiri *et al.*, 2016) ^[1].

Results and Discussion

Phytochemical Analysis

The preliminary phytochemical analysis results of *F. schweinfurthii* (aqueous and ethanol extracts) were recorded (Table 1). *F. schweinfurthii* Shoot and root extracts contains Alkaloids, Starch, Protein, Tannin, Flavonoid, Terpenoid, Carbohydrates, Lignin and Phenols.

Antibacterial Assay by Agar well diffusion method

The results of anti-bacterial activities of crude extracts of *F*. schweinfurthii were screened by Agar well diffusion method against 5 microorganisms including 3 Gram +ve and 2 Gram -ve the mean values of zone of inhibition were recorded (Table 2, 3). Aqueous 100 μ l shoot extract showed slight inhibitory activity against *E. coli* (12.6±0.68), *K. pneumonia* (15±0.74), *S. aureus* (10.6±0.62), *P.vulgaris* (12±0.66) and *B. subtilis* (10.3±0.61). Methanol 100 μ l shoot extract showed significant inhibitory activity against *E. coli* (18.7±0.83), *K. pneumonia* (19±0.83), *S. aureus* (15.6±0.76) *P.vulgaris* (14.6±0.73) and *B. subtilis* (13±0.71). The Benzene 100 μ l shoot extract showed moderate inhibitory activity against *E. coli* (11.3±0.64), *K. pneumonia* (10.7±0.63), *S. aureus* (14±0.72), *P.vulgaris* (N.D.) and *B. subtilis* (12±0.66)(Fig. 2-6).

Aqueous 100 µl root extract showed slight inhibitory activity against *E. coli* (11.6±0.65), *K. pneumonia* (13.6±0.71), *S. aureus* (10±0.60), *P.vulgaris* (11.3±0.64) and *B. subtilis* (N. D.). Methanol 100 µl root extract showed significant inhibitory activity against *E. coli* (17.6±0.80), *K. pneumonia* (17±0.79), *S. aureus* (17±0.79), *P.vulgaris* (16.3±0.77) and *B. subtilis* (14±0.72).

The Benzene 100 μ l root extract showed moderate inhibitory activity against *E. coli* (N.D.), *K. pneumonia* (16.3±0.77), *S. aureus* (9.3±0.58), *P.vulgaris* (11.3±0.64) and *B. subtilis* (12.3±0.67) (Fig. 3-7).

The activity was found to be more on the Gram positive organism (K. *pneumoniae*) than the Gram negative organisms

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tested. The Methanol extract of the plant was found to inhibit the organism only at the higher concentration $(100\mu l)$. The activity of the extract can be attributed to the presence of higher polar constituents as the methanol and aqueous extracts were found to be more active than the benzene, petroleum ether, chloroform and toluene extract. Gentamicin is used as Positive control against *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Staphylococcus aureus* showed the zone of inhibition in between 33±0.23mm to 39±0.43mm.



Fig 1: Habitat- F. schweinfurthii

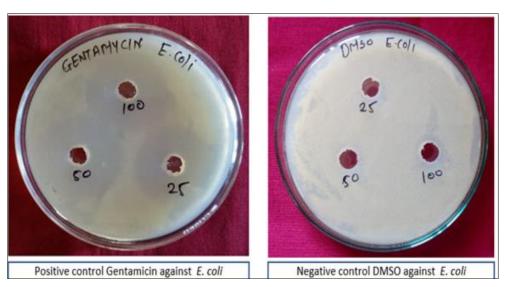


Fig 2: Antimicrobial activity of against Positive and negative Control

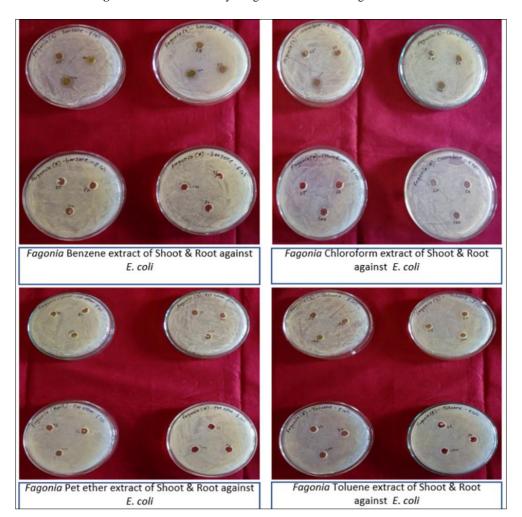


Fig 3a: Antimicrobial activity of against Positive and negative Control

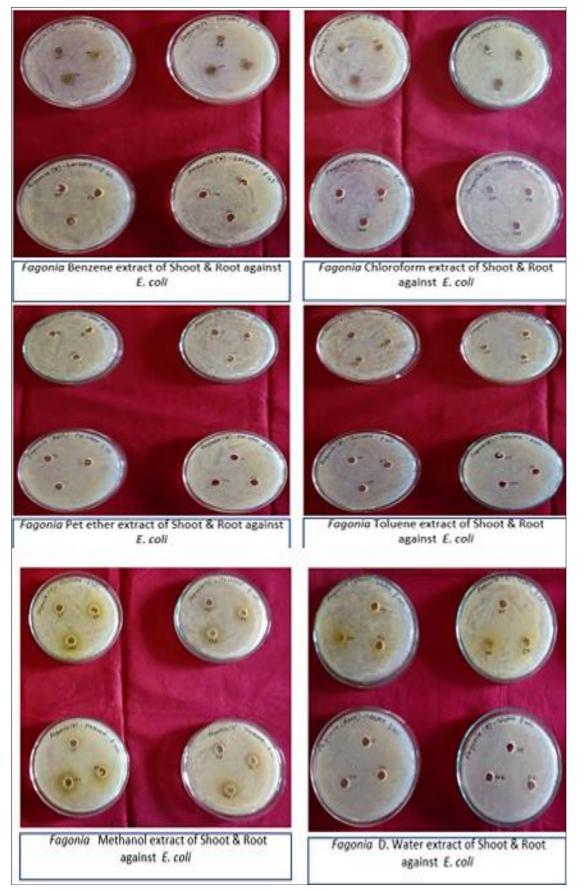


Fig 3b: Antimicrobial activity of against E. coli

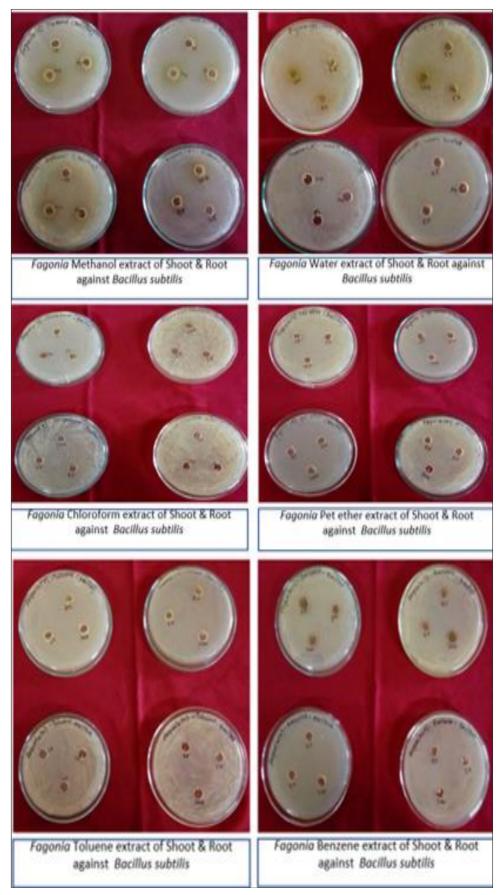


Fig 4: Antimicrobial activity of against B. subtilis

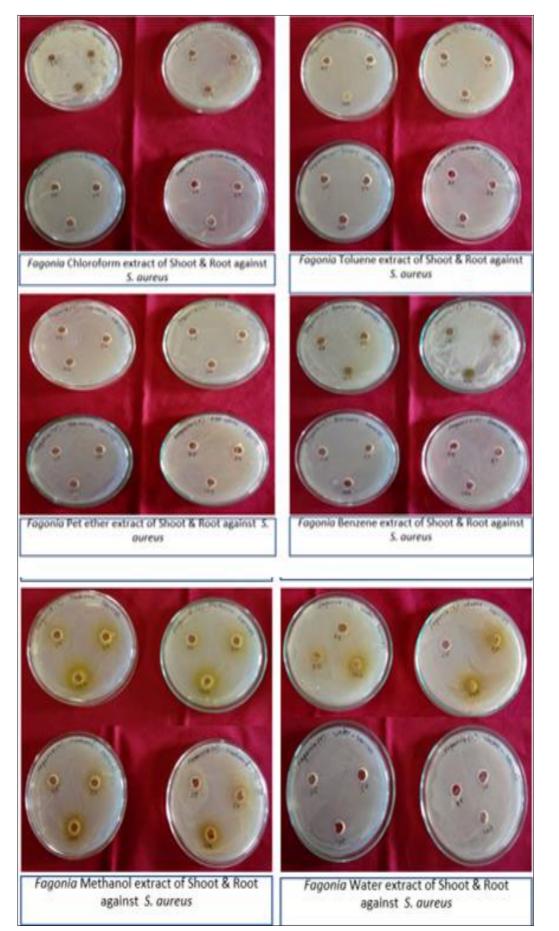


Fig 5: Antimicrobial activity of against S. aureus

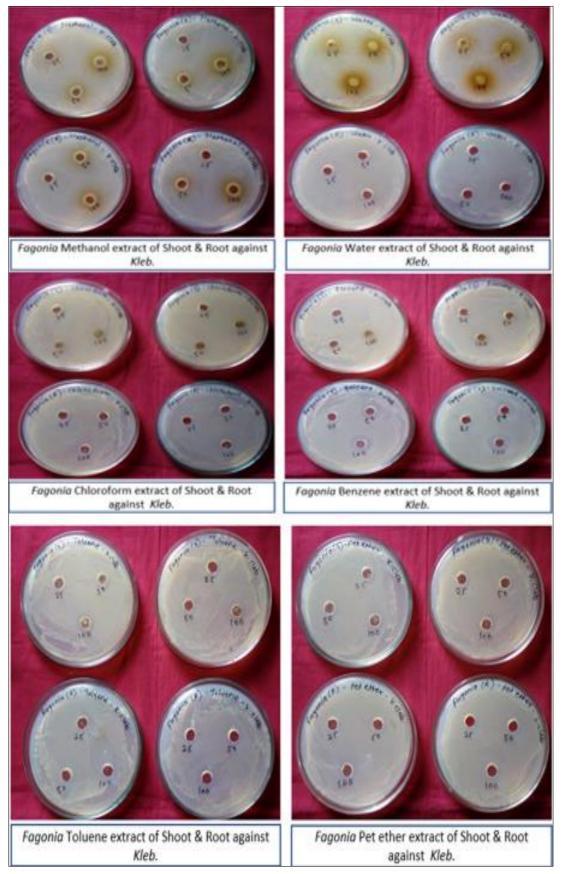


Fig 6: Antimicrobial activity of against Kleb

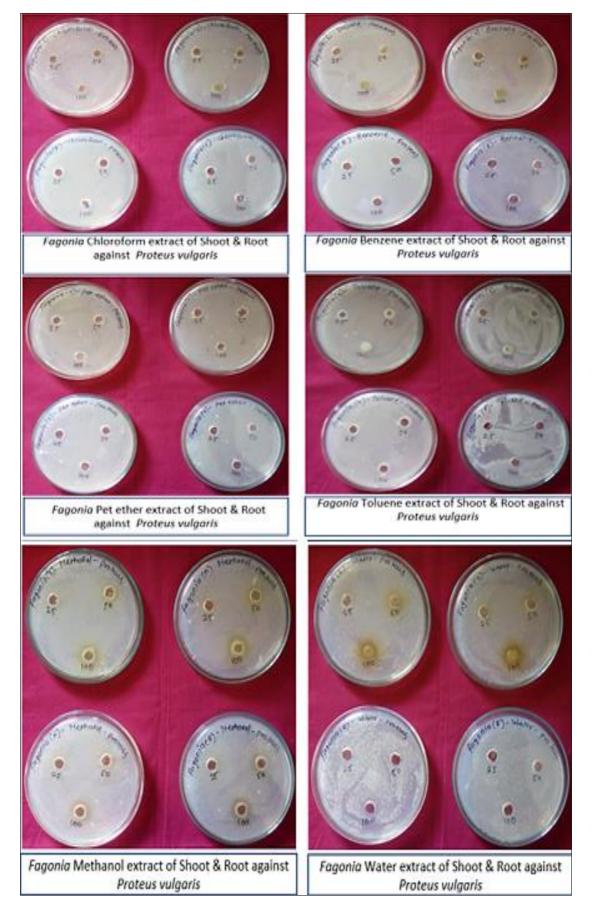


Fig 7: Antimicrobial activity of against P. vulgaris

Phytochemicals	Result			
•	Shoot parts extract		Root extract.	
	A.E.	E.E.	A.E.	E.E.
Alkaloids	+	+	+	+
Starch	+	+	+	+
Protein	+	+	+	+
Tannin	+	+	+	+
Saponin	+	-	+	-
Flavonoid	+	+	+	+
Free Amino Acid	+	-	+	-
Terpenoid	+	+	+	+
Carbohydrates	+	+	+	+
Lignin	+	+	+	+
Phenols	+	+	+	+

Plant Extract of Shoot	Concentration (µl)	<i>E. coli</i> zone of Inhibition in mm ±SE	<i>K. pneumoniae</i> zone of inhibition in mm ±SE	S. aureus zone of inhibition in mm ±SE	Proteus vulgaris zone of inhibition in mm ±SE	Bacillus subtilis zone of inhibition in mm ±SE
Distilled Water	25 µl	9.01±0.57	10±0.60	N.D.	8.3±0.55	N.D.
	50 µl	10.6±0.62	12±0.66	N.D.	10±0.61	N.D.
	100 µl	12.6±0.68	15±0.74	10.6±0.62	12±0.66	10.3±0.61
Methanol	25 µl	11.7±0.65	10±0.62	11.6±0.65	9.60±0.59	10±0.60
	50 µl	14.7±0.73	15±0.74	13.6±0.71	11.6±0.65	12±0.66
	100 µl	18.7 ± 0.83	19±0.83	15.6±0.76	14.6±0.73	13±0.71
Benzene	25 µl	N.D.	N.D.	8.6±0.56		8.6±0.56
	50 µl	6.11±0.59	N.D.	11±0.63	N.D.	10±0.62
	100 µl	11.3±0.64	10.7±0.63	14±0.72		12±0.66
Chloroform	25 µl	7.6±0.53	N.D.	N.D.	N.D.	N.D.
	50 µl	8.6±0.56				
	100 µl	10±0.61				
Pet ether	25 µl	N.D.	N.D.	N.D.	N.D.	N.D.
	50 µl					
	100 µl					
Toluene	25 µl	N.D.	N.D.	N.D.	8.6±0.56	9.3±0.58
	50 µl		N.D.		9.3±0.58	11±0.63
	100 µl		11.6±0.65		10.3±0.61	12±0.68

N.D. = Not Detected

Table 3: Zone of inhibition exhibited by bacteria with different solvents of *F. chweinfurthii* Root extract.

Plant Extract of Root	Concentration (µl)	<i>E. coli</i> zone of Inhibition in mm ±SE	<i>K. pneumonia</i> zone of inhibition in mm ±SE	S. aureus zone of inhibition in mm ±SE	P. vulgaris zone of inhibition in mm ±SE	B. subtilis zone of inhibition in mm ±SE
Distilled Water	25 µl	3.3±0.55	9.3±0.58	N.D.	8.6±0.56	
	50 µl	9.6±0.59	12±0.66	N.D.	10±0.60	N.D.
	100 µl	11.6±0.65	13.6±0.71	10±0.60	11.3±0.64	
Methanol	25 µl	11.6±0.65	11±0.63	12±0.66	9.6±0.59	10.6±0.62
	50 µl	14.6±0.73	15±0.74	14.3±0.72	12±0.66	11.6±0.65
	100 µl	17.6±0.80	17±0.79	17±0.79	16.3±0.77	14±0.72
Benzene	25 µl	N.D.	N.D.	N.D.	8±0.54	9.3±0.58
	50 µl		14±0.72	N.D.	9.3±0.58	11±0.63
	100 µl		16.3±0.77	9.3±0.58	11.3±0.64	12.3±0.67
Chloroform	25 µl	N.D.	N.D.	N.D.	N.D.	N.D.
	50 µl				N.D.	
	100 µl				10±0.60	
Pet ether	25 µl	N.D.	N.D.	N.D.	N.D.	N.D.
	50 µl		N.D.			
	100 µl		10.6±0.62			
Toluene	25 µl	N.D.	N.D.	N.D.	9±0.57	N.D.
	50 µl		N.D.		10±0.60	
	100 µl		10.6±0.62		11±0.63	

N.D. = Not Detected

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