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Evaluation of the Gelling Property of Some Local Plants Seeds as Alternative Gelling Agents to Agar-Based Microbial Culture Media

Udofa, Edidiong Jumbo[1], Thomas, Paul Sunday[2] Alozie, Mfonobong Favour[3], and Nkanang, Abigail Johnny[4], Ekong, Ubong Samuel[5]

[1,3,5]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria.

[2] Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria.

[4]Department of Microbiology, Faculty of Sciences, University of Uyo, Akwa Ibom State, Nigeria.

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ABSTRACT

Agar, an expensive gelling agent for culturing microbes affects holistic microbiological and pharmaceutical usage. Hence, plants used as thickening agents should be investigated. This work aims to evaluate the gelling properties of the seeds of Brachystegia eurycoma, Mucuna sloanei, Detarium microcarpum and Cucumeropsis mannii, as alternative gelling agents to agar in microbial culture media. The analyses were investigated in accordance with standard procedures and their modifications by pulverization, bleaching and physicochemical methods. The 3 plants showed relative gelling property when compared to that of agar-based media except for Cucumeropsis mannii which did not exhibit any gelling property at all concentrations. At the least (0.056g/mL) and highest (0.081g/mL) concentrations with equivalent gelling activity to agar at 0.015g/mL as the standard, B. eurycoma exhibited significant ($p<0.05$) gelling property in more concentrations compared to D. microcarpum and M. sloanei. Furthermore, there was improved gelling activities of the plants after treatment with 3% sodium hypochlorite especially with 250mm than 500mm sieve sizes. Homogeneity, consistency, surface-streaking, transparency, smoothness, hardness, friability of nutrient and sabouraud dextrose broths formulated with different seeds powder showed significant ($P<0.05$) disparities; while gelling strength (0.0325-0.13mg/ml), time (2-26 minutes) and colour (amber-light yellow) as well as pH (4.8-6.4) were significantly ($P<0.05$) comparable to that of agar with 0.0028-0.0056mg/ml, 10-15 minutes, amber colour, 5.6 and 6.6 as pH respectively. From this study, B. eurycoma exhibited good gelling properties followed by D. microcarpum and M. sloanei that are worth investigating further as possible alternative gelling agent to agar for formulation of microbial culture media.

Keywords: Alternative-Gelling-Property, B. eurycoma, D. microcarpum, M. sloanei, Microbial-Culture-Media.

INTRODUCTION

There has been a paradigm shift in technology advancement in industrial, pharmaceutical and microbiological development arising from the involvement and utilization of local plants as natural resources especially in developing countries. These local plants with abundant bio metabolic or secondary compounds of varying attributes and activities also possessed the ability to form gel with other characteristic whose usefulness can never be over-emphasized. Gelling agents (GA) are polysaccharides derived from different sources, for solidification and stability as amongst the important ingredients in media formulations for plant tissue culture in microbiological field, rheological and delivery applications in pharmaceutical technology and other spheres of practice. However, the type of GA used can influence the growth of the tissue in the culture. Additionally, purity (level and composition of impurity) and cost of the GA are very vital in any research and operations. Different types of GA exist but the most conventional one is agar. Besides, the expansion of this activity is

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basically hampered by the high cost of plants regenerated through tissue culture. But agar adds to the cost of regenerated plants (Ezekiel, 2010). Several attempts in the past have been made with few colloidal polysaccharides of microbial or plant origin as potential, suitable and cheaper alternative to agar as gelling agent for microbial and plant tissue culture media (Jain et al., 1997, 2005; Babbar and Jain 1998, 2002, 2005 and 2006). However, despite all these having a distinct cost advantage over agar, none is likely to be used as routinely as agar because of some inherent drawbacks Agar is a mixture of two components, the linear polysaccharide agarose and a heterogeneous mixture of smaller molecules called agaropectin (Rafael and Fernando, 1987; Williams and Philips, 2000) extracted from seaweeds, belonging to the Rhodophyta (red algae) (Edward, 1871), a gelatinous and inert substance devoid of any content and used in culture media as a gelling agent. But there are some indigenous or local plants that are easily accessible, readily available and very cheap, known to be used as soup thickeners and can be evaluated for gel formation as alternative to agar (Ukachukwu et al., 2002; Ajuru and Okoli, 2013). These plants amongst others include Mucuna sloanei Fawc and Rendle (Fabaceae), Brachystegia eurycoma Harm (Fabaceae), Detarium microcolumn Guill and Perr (Fabaceae) and Cucumeropsis mannii Naudin (Cucurbitaceae). Mucuna is a genus of about 150 accepted species of annual and perennial legumes of the family Fabaceae (AGP, 2016) and of pan-tropical distribution with India as one of the natural centers in the world (Eilitta, et al., 2002). It is widespread in Africa from Sierra Leone East to DR Congo, and South to Angola, as well as the Caribbean region, Tropical America and Islands of the Pacific Ocean (Jansen, 2005).

Figure 1: Mucuna sloanei with fruits (left) and Seeds (right)

In Nigeria, Mucuna sloanei is called different names such as; ‘ukpo’ by the Igbos, ‘karasuu’ by the Hausas, ‘yerepe’ by the Yorubas and ‘ibaba’ by the Annang, Efik and Ibibio. It is commonly called ‘horse eye bean’ in English (Obochi et al., 2007; Nwosu, 2012), The plants have been reported to possess useful constituents of high medicinal value of human and veterinary importance and also constitute an important raw material in Ayurvedic and folk medicines (Ojiako, et al., 2012; Ejere et al., 2015;). The seeds are rich in alkaloids as well as antioxidants, antitumor and antibacterial compounds (Ekwe et al., 2016). In the South Eastern part of Nigeria, M. sloaneii is used as thickeners in local delicacies. However, it is under-utilized in formulations and medicine (Ukachukwu et al., 2002). Brachystegia eurycoma is a seasonal legume known for its ethno-medicinal and nutritional values, popular among the people of the South Eastern part of Nigeria (Okoli, et al., 2015). It is an economic and woody plant belonging to the family Fabaceae and mostly found in the rain forest zone with attractive description (APG, 2016; Ndukwu, et al., 2017).

Figure 2: Brachystegia eurycoma tree (left) and Seeds (right)

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In Nigeria B. euyrocoma is called “achi” in Igbo, “akalado” or “eku” in Yoruba, “akpakpa” or “taura” in Hausa, “apauan” by the Ijaws, “dewen” in Benin and “odukpa” in Efik, Ibibio and Annang (Obochi, et al., 2007). B. eurycoma is known to exhibit variety of activities (Ajuru and Okoli, 2013; Onyeso, et al., 2016; Ndukwu, et al., 2017). It is used in the South Eastern part of Nigeria as thickeners in local delicacies (Ajuru and Okoli, 2013) However, B. eurycoma has been grossly under-utilized despite the promise that it holds for food and drug development.

Detarium microcolumn (Guill and Perr) is an African tree known as sweet detar, sweet dattock or tallow tree belonging to the family Fabaceae (AGP, 2016; Gaisberger et al., 2017). It is an under-utilized species that grows naturally in the drier regions of West and Central African; from Senegal and Gambia east to Sudan and widely distributed in the semi-arid sub-Saharan African which include Benin, Burkina Faso, Nigeria etc., (Dayamba, et al., 2016).

Figure 3: Detarium microcarpum tree (left) and Seeds (right).

D. microcarpum is one of the three species (D. macrocarpon and D. senegalense) of Detarium genus. D. microcarpum is called “taura” in Hausa, “kanuri” in Fulani, “algacidal”, “Etsuko” in Tiv, “eyed” in Yoruba, “ofor” in Igbo, Annang, Ibibio and Efik, (Iwu, 1993). D. microcarpum is known to contain bio-active and other substances for variety of uses. Despites its versatility (the numerous ethno-medical, industrial, pharmacological, etc. uses), this specie remains under-utilized especially it’s thickening property. Hence the need for its inclusion for further research works. Cucumeropsis manni is a species of melon native to Tropical Africa West of the Great Rift Valley (Edunjobi and Adebisi, 2004; Hanno and Susanne, 2010) from the family, Cucurbitaceae (APG, 2006) where it is grown for food and as a source of oil.

Figure 4: Cucumeropsis manni with fruit (left) and Seeds (right).

In Nigeria, this plant is called “Ahu-ilu/Elegushi” in Igbo, “Egusi” in Yoruba, “Agushi” in Hausa, “Ikon” in Annang, Efik and Ibibio, and white seed melon in English (Obute and Adubor, 2007). The proximate, phytochemical and elemental parameters of C. mannii which are responsible for its numerous activities have been reported (Badifu and Ogunsua, 1991; Abiodun and Adeleke 2010). It is widely used as a thickener in soups in Nigeria, Cameroon, Benin, and Cote d’Ivoire (Koffi et al., 2008; Adewale and Mozie, 2010). Despite its agronomic, cultural and nutritional importance, the plant lacks attention from research and development,

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hence it is so categorized under the orphan crops of Africa (Adewale and Mozie, 2010; Abiodun and Adeleke, 2010). Therefore, this work seeks to evaluate the gelling properties of the seeds of B. eurycoma, M. sloanei, D. microcarpum and C. mannii, as alternative gelling agents to agar in agar-based microbial culture media.

Statement of the Problem

The current microbial culture media is characterized by some limitations including increasing cost among other. This has seriously affected students' work, effective research, and hindered holistic learning. This has post serious problem for developing countries, including Nigeria and other African countries (Adesemeye and Adedire, 2005). Besides, culture-based approaches for isolation also failed to determine the predominant microorganisms in nature that could not be cultivated using standard techniques/media (Amann and Ludwig, 1995). Furthermore, the diversity of cell lines and the involvement of a large number of media components are interdependent on others.

This adds another layer of complexity because of the complexity of cellular metabolites. Nevertheless, there are locally source and readily available plants seed with gelling properties that could be similar to agar, that have not yet been exploited in microbial culture media formulation. Hence, the need to evaluate these plants for its gelling properties in view of formulating new and/or alternative media with low cost.

MATERIALS AND METHODS

Seed Pulverization: The seeds of the different plants were pulverized into powder using hammer mill machine. The pulverized seeds were sieved using sieving filter of sizes 250 and 500 mm. The fine seed powders were separately stored in an air-tight container until needed for use.

Gelling Formation from Pulverized Plants-Seeds Before Treatment: Gel solutions were prepared by adding varying amount (0.7-8.4 g) of pulverized plants seeds of B. eurycoma, M. sloanei, D. microcarpum and C. mannii to distilled water (25 -150 mL) with stirring for homogenous mix. The pH of the solutions was checked using pH litmus paper, for acidity, alkalinity or neutrality. The solutions were sterilized by autoclaving (Dixons, ST19T) at 121 ^{o}C for 15 -20 minutes under the pressure of 15 Ibs/inch, allowed to cool to a temperature of about 45 ^{o}C and then dispensed into labelled sterile petri dishes. All the prepared gels were then subjected to various evaluation tests for their morphological characteristics (homogeneity, presence of particles consistency, transparency/clarity, smoothness, hardness, friability, surface streaking potential, gelling strength and time and color) using visual/physical methods. These were compared to that of agar-based NA and SDA as standards.

Treatment of Pulverized Plants-Seeds: This was carried out according to standard biochemical methods to evaluate possibility of activity change in color, particle size dissolution, and other gelling parameters.

Gelling Formation from Pulverized Plants-Seeds After Treatment: The same procedure for gelling of pulverized plants-seeds before treatment was followed**.**

Statistical Data Analysis: Results obtained were statistically analyzed and presented using bar-charts.

RESULTS AND DISCUSSIONS

Table 1: Gelling Capacity of Pulverized Seeds Before Treatment

Vol(mL)	**Wt.(g)**	**Gelling Capacity of 250 mm Sieve Size at Different Time (min)**			
		B. eurycoma	M. sloanei	D. microcarpum	C. mannii
25	0.700	Not gelled (>60)	**Not gelled (60.0)**	Not gelled (60.0)	Not gelled (>60.0)
	1.400	Not gelled (52.0)	Gelled (30.0) *	Gelled (20.0) **	Not gelled (>60.0)

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	1.625	Gelled (16.0) **	Gelled (very good) (16.0)	Gelled (15.0) **	Not gelled (>60.0)
	3.250	Gelled (12.0) **	Gels on sterilization (3.0)	Gelled (10.0) **	Not gelled (>60.0)
50	0.700	Not gelled (>60)	Not gelled (>60.0)	Not gelled (52.0)	Not gelled (>60.0)
	1.400	Gelled (15) **	Not gelled (58.0)	Not gelled (48.0)	Not gelled (>60.0)
	2.800	Gelled (8.0) **	Gelled (very good) (17.0) **	Gelled (34.0) *	Not gelled (>60.0)
	1.625	Partial gelling (32.0)	Gelled (24.0) *	Gelled (27.0) *	Not gelled (>60.0)
	3.250	Gelled (2.0) **	Gelled (very good) (15.0) **	Gels on sterilization 5.0)	Not gelled (>60.0)
	6.500	Gels on sterilization 1.0	Gels on sterilization (2.0)	Gels on sterilization (2.0)	Not gelled (>60.0)
100	2.800	Partial gelling (58.0)	Not gelled (55.0)	Not gelled (55.0)	Not gelled (>60.0)
	3.250	Partial Gelling (47.0)	Partial Gelling (47.0)	Gelled (25.0) *	Not gelled (>60.0)
	6.500	Partial gelling (40.0)	Gelled (40.0) *	Gelled (20.0) **	Not gelled (>60.0)

Key: Vol. (volume of distilled water), Wt. (weight) *(gelling capacity), **(gelling property comparable to controls)

Table 2: Gelling Capacity of Pulverized Seeds Before Treatment

Vol. (mL)	**Weight (g)**	**Gelling Capacity of 500 mm Sieve Size at Different Time (min)**			
		B. eurycoma	M. sloanei	D. microcarpum	C. mannii
25	0.700	Not gelled (>60)	**Not gelled (60.0)**	Not gelled (60.0)	Not gelled (>60.0)
	1.400	Partial gelling (60.0)	Partial gelling (>60.0)	Gelled (26.0) **	Not gelled (>60.0)
	1.625	Gelled (20.0) **	Partial gelling (>60.0)	Gelled (20.0) **	Not gelled (>60.0)
	3.250	Gelled (15.0) **	Gelled (10.0)	Gelled (14.0) **	Not gelled (>60.0)
50	0.700	Not gelled (60)	Not gelled (>60.0)	Not gelled (>60.0)	Not gelled (>60.0)
	1.400	Partial gelling (60.0)	Not gelled (>60.0)	Not gelled (>60.0)	Not gelled (>60.0)
	2.800	Gelled (5.0) **	Gelled (25.0) **	Gelled (25.0) *	Not gelled (>60.0)
	1.625	Partial gelling (60.0)	Gelled (32.0) *	Gelled (32.0) *	Not gelled (>60.0)
	3.250	Gelled (5.5) **	Gels on sterilization (5.0)	Gelson sterilization (7.0)	Not gelled (>60.0)
	6.500	Gelled (15.0) **	Gels on sterilization	Gels on	Not gelled (>60.0)

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			(3.0)	sterilization (3.0)	
100	2.800	Partial gelling (60.0)	Not gelled (>60.0)	Not gelled (60.0)	Not gelled (>60.0)
	3.250	Partial Gelling (60.0)	Not gelled (60.0)	Gelled (30.0) *	Not gelled (>60.0)
	6.500	Partial gelling (60.0)	Partial gelling (40.0)	Gelled (22.0) **	Not gelled (>60.0)

Key: Vol. (volume of distilled water), * (gelling capacity), ** (gelling property comparable to controls)

Tables 1 and 2 showed gelling capacity with mesh sieve sizes 250 and 500 mm at varying concentrations and time of the different pulverized plants seeds of M. sloanei, B. eurycoma and D. microcarpum except for C. mannii which did not gel at all. The activity from these plants was observed as gelled, partial gelling, gelled on sterilization and not gelled. The 2 sieve sizes 250 and 500 mm also showed relative gelling results at different time intervals. But 250 mm gave a better gelling activity at shorter time compared to that of 500 mm sieve size. The lowest and highest gelling concentrations with distilled water without any addition of nutritive component were observed in M. sloanei at 1.400 g/25 mL (0.056 g/mL), B. eurycoma 1.625 g/25 mL (0.065 g/mL) and 3.250 g/100 mL (0.0325 g/mL) while D. microcarpum only gelled at 1.625 g/50 mL (0.0325 g/mL) respectively. Also, at 3.250 g/25 mL (0.13 g/mL), M. sloanei gelled on sterilization (before removing from the autoclave) but that of D. micropump was at 3.25 g/50 mL (0.065 g/mL) and 6.500 g/50mL (0.13 g/mL). B. eurycoma, D. microcarpum and M. sloanei. This suggest that a gelling concentration could be achieved by varying the concentrations between 1.400 g/25 mL (0.056 g/mL) and 6.25 g/100mL (0.0625 g/mL), less or more to have a suitable gelling specific for the three plants. This gelling or thickening property of the pulverized plants seeds of M. sloanei, B. eurycoma and D. microcarpum is in line with already published work Ukachukwu et al., 2002; Onweluzo, et al., 2004; Ameh, et al., 2010; Adedeji, et al., 2012; Ajuru and Okoli, 2013; Aviara, 2015). But the concentrations for C. mannii in this research did not align with the thickening property as recorded by Adewale and Mozie, (2010). The various or varying concentrations are also selectively at variant with agar-based gelling capacity at same concentrations (Marcus, 2013).

Table 3: Gelling Capacity of Pulverized Seeds After Treatment

Vol (mL)	Weight (g)	Gelling Capacity of 250 mm Sieve Size at Different Time (min)			
		B. eurycoma	M. sloanei	D. microcarpum	C. mannii
25	0.700	Not gelled (>60)	Not gelled (60.0)	Not gelled (60.0)	Not gelled (>60.0)
	1.400	Partial gelling (47.0)	Partial gelling (60.0)	Gelled (25.0) **	Not gelled (>60.0)
	1.625	Gelled (21.0) **	Gelled (19.0) **	Gelled (20.0) **	Not gelled (>60.0)
	3.250	Gelled (15.0) **	Gelled (10.0) **	Gelled (14.0) **	Not gelled (>60.0)
50	0.700	Not gelled (>60)	Not gelled (>60.0)	Not gelled (60.0)	Not gelled (>60.0)
	1.400	Partial gelling (60.0)	Not gelled (60.0)	Not gelled (60.0)	Not gelled (>60.0)
	2.800	Gelled (5.0) **	Gelled (25.0) **	Gelled (25.0) *	Not gelled (>60.0)
	1.625	Partial gelling (35.0)	Gelled (32.0) *	Gelled (32.0) *	Not gelled (>60.0)
	3.250	Gelled (5.5) **	Gelled (5.0) **	Gelled (7.0) *	Not gelled (>60.0)
	6.500	Gelled (15.0) **	Gelled (3.0)	Gelled (3.0) **	Not gelled (>60.0)
100	2.800	Partial gelling (60.0)	Not gelled (60.0)	Not gelled (55.0)	Not gelled (>60.0)
	3.250	Partial Gelling (60.0)	Not gelled (60.0)	Gelled (30.0) *	Not gelled (>60.0)
	6.500	Partial gelling (60.0)	Partial gelling (60.0) *	Gelled (22.0) *	Not gelled (>60.0)

Key: Vol (volume of distilled water), * (gelling capacity), ** (gelling property comparable to controls)

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Table 4: Gelling Capacity of Pulverized Seeds After Treatment

Vol (mL)	Weight (g)	Gelling Capacity of 500 mm Sieve Size at Different Time (min)			
		B. eurycoma	M. sloanei	D. microcarpum	C. mannii
25	0.700	Not gelled (>60)	Not gelled (60.0**)**	Not gelled (60.0)	Not gelled (>60.0)
	1.400	Not gelled (>60.0)	Gelled (30.0) *	Gelled (20.0) **	Not gelled (>60.0)
	1.625	Gelled (16.0) **	Gelled (very good) (16.0) **	Gelled (15.0) **	Not gelled (>60.0)
	3.250	Gelled (12.0) **	Gelled on sterilization (3.0)	Gelled (10.0) **	Not gelled (>60.0)
50	0.700	Not gelled (>60)	Not gelled (>60.0)	Not gelled (>60.0)	Not gelled (>60.0)
	1.400	Gelled (15.0) **	Not gelled (>60.0)	Not gelled (>60.0)	Not gelled (>60.0)
	2.800	Gelled (8.0) **	Gelled (very good) (17.0) **	Gelled (34.0) *	Not gelled (>60.0)
	1.625	Partial gelling (60.0)	Gelled (24.0) *	Gelled (27.0) *	Not gelled (>60.0)
	3.250	Gelled (2.0) **	Gelled (very good) (13.0) **	Gelled (5.0) **	Not gelled (>60.0)
	6.500	Gelled (3.0) **	Gelled (7.0)	Gelled (2.0) **	Not gelled (>60.0)
100	2.800	Partial gelling (60.0)	Not gelled (60.0)	Not gelled (60.0)	Not gelled (>60.0)
	3.250	Partial Gelling (60.0)	Partial gelling (60.0)	Gelled (25.0) *	Not gelled (>60.0)
	6.500	Partial gelling (60.0)	Gelled (40.0) *	Gelled (20.0) **	Not gelled (>60.0)

Key: Vol. (volume of distilled water), *(gelling capacity), **(gelling property comparable to controls).

The results observed after the treatment/bleaching showed slight improved in colour; and gelling activity which changed from gel on sterilization to gelled (*) on the 3 pulverized plants seeds (Table 3). This could be attributed to incomplete washing or the effect of the bleaching agent on the pulverized seeds or reduction in the plants phytochemical, proximate and elemental components known to be present in the plants (Onweluzo, et al., 1994 ; Onweluzo, et al.,1999; Akpata and Miachi, 2001; Ijeh et al., 2004; Cavin, et al., 2006; Sridhar and Rajeev, 2007; Okwu and Okoro, 2007; Ameh, et al., 2010; Barmina, et al., 2012; Ojiako, et al., 2012; Ajuru and Okoli, 2013; Igwe and Okwu, 2013; Bolanle et al., 2014; Johnny et al., 2014; Ejere et al., 2015; Ekwe et al., 2016; Onyeso, et al., 2016; Ndukwu, et al., 2017). D. microcarpum and B. eurycoma invariably had significant ($p<0.05$) gelling strength, smoothness, surface streaking, colour and consistency compared to the pulverized plants-seeds without treatment. M. sloanei showed slight change in colour from black to light grey. This also confirmed the possibility of using B. eurycoma, D. microcarpum and M. sloanei as agar agar replacement for microbial culture media formulation. The differences observed could be due to the presence of particle size and dissolution for homogenous solution. But the gelling time was between 30 and 40 minutes as against 18 and 25 minutes for that of controls (NA and SDA). M. sloanei had the longest gelling time of 40 minutes. Formulation with Nutrient broth (NB) showed less gelling time (30 mins) compared to SDB (35 mins) for B. eurycoma; formulation with Sabouraud Dextrose broth (SDB) also showed less gelling time (37 mins) compared to NB (40 mins) for M. sloanei whereas SDB formulation base showed less gelling time (32 mins) compared to NB (35 mins) for D. microcarpum, respectively. Therefore, the nutritive based or broth for this microbial culture media formulation follows; SDB > NB. Using Nutrient and Sabouraud Dextrose broths was to determined or ascertained which broth will favor an ideal and standard formulated microbial culture media and best activity after formulation. The effect of particle size, complete dissolution of the pulverized plants seeds and homogenous mix before and after sterilization may have contributed to the delayed in gelling time and other differences observed in the morphological parameters. This is a challenge that needs to be solved as spotted in the course of this novel work. The bar-charts below showed the results of the gelling capacities of these pulverized plants seeds.

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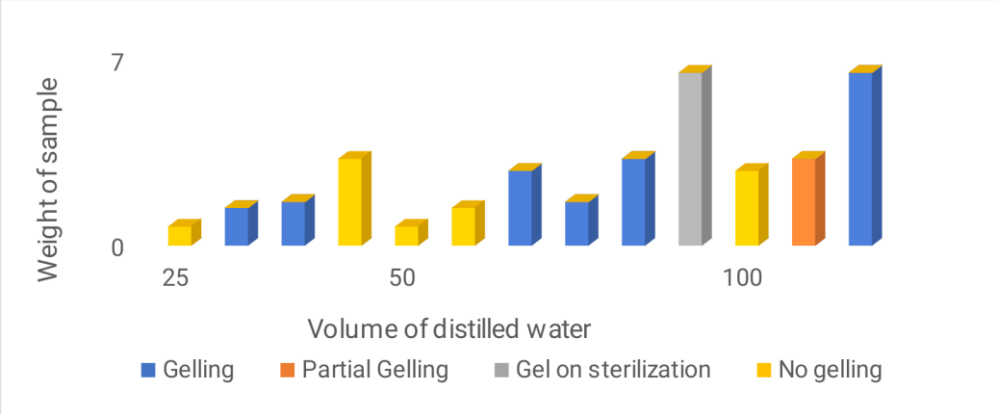

Figure 5: Gelling Property of 250 mm sieve size Pulverized Plant Seeds of Mucuna sloaneii

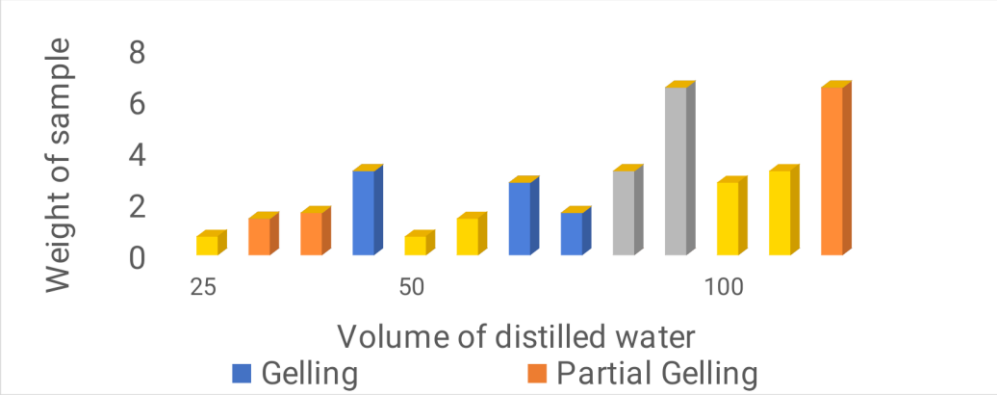

Figure 6: Gelling Property of 500 mm sieve size Pulverized Plant Seeds of Mucuna sloaneii

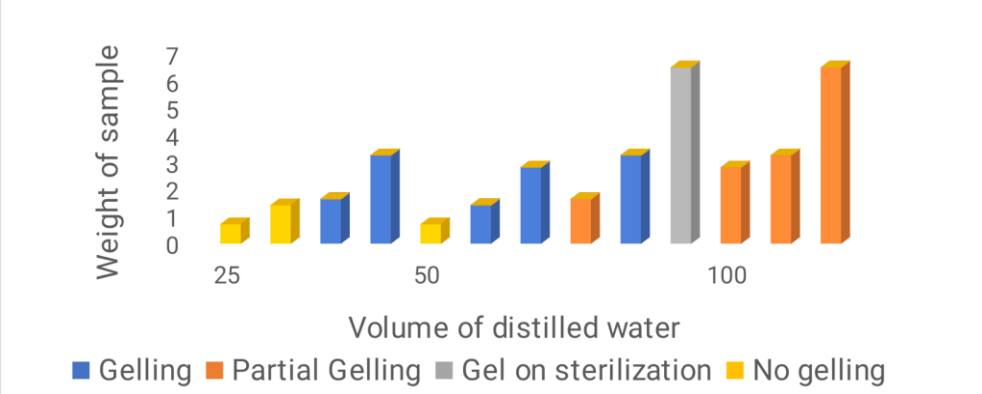

Figure 7: Gelling Property of 250 mm sieve size Pulverized Plant Seeds of Brachystegia eurycoma

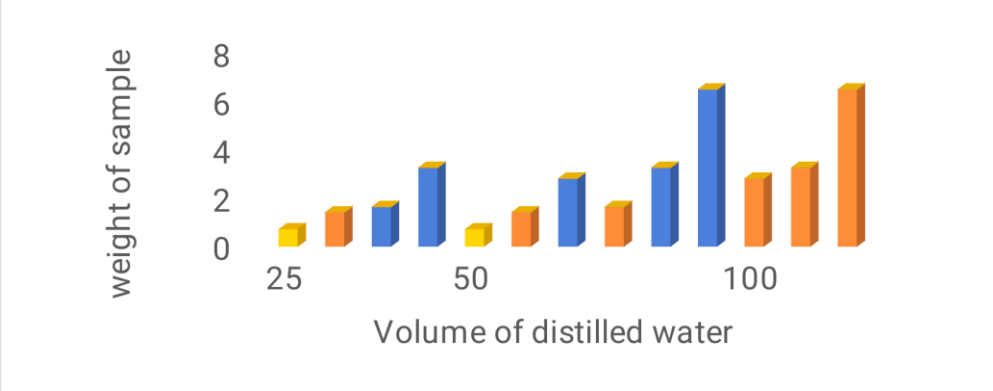

Figure 8: Gelling Property of 500 mm sieve size Pulverized Plant Seeds of Brachystegia eurycoma

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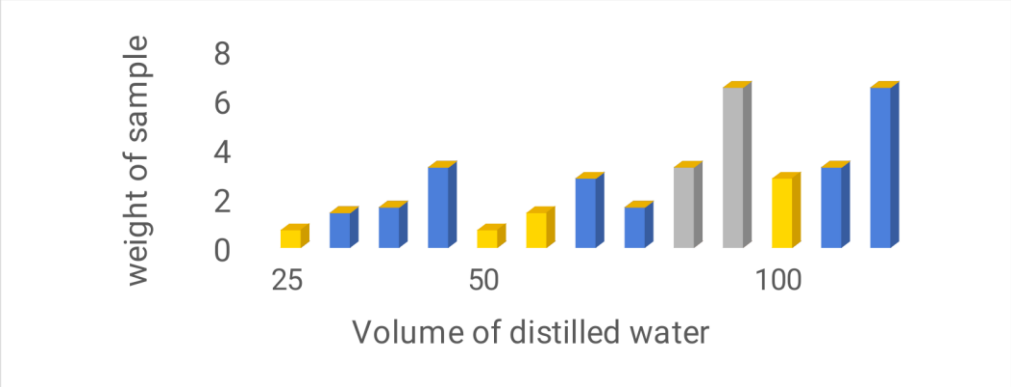

Figure 9: Gelling Property of 250 mm sieve size Pulverized Plant Seeds of Deturium microcarpum

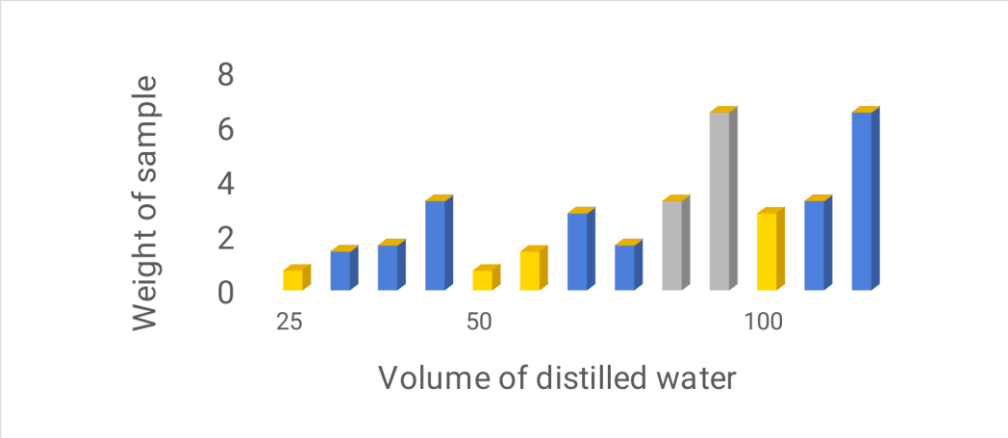

Figure 10: Gelling Property of 250 mm sieve size Pulverized Plant Seeds of Deturium microcapum

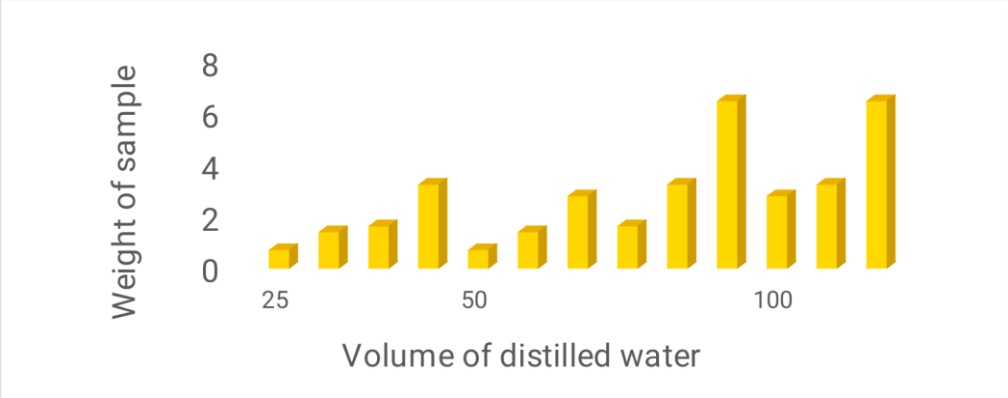

Figure 11: Gelling property of 250- and 500-mm sieve sizes of Pulverized Plant seeds of C. mannii

Table 5: Morphological Parameters of Gelled Pulverized Plants-Seeds with Standard Broths

Parameters/	B. eurycoma		M. sloanei		D. microcapum		Control	
Weight g/mL	0.028	0.0654	0.028	0.0654	0.028	0.0654	NA	SDA
Homogeneity	Not	Not	Not	Not	Slightly	Slightly	Very	Very
Clarity	Not	Not	Not	Not	Not very	Not very	Very	Very
Particles	Yes	Yes	Yes	Yes	Slightly	Slightly	None	None
Consistency	Less	Less	Less	Less	Less	Less	Very	Very

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Transparency	Slightly	Very	Slightly	Slightly	Slightly	Slightly	Very	Very
Hardness	Not too Hard	Not too hard	Not too Hard	Not too hard	Not too Hard	Not too Hard	Not too Hard	Not too hard
Smoothness	Fairly smooth	Fairly smooth	Fairly smooth	Fairly smooth	Very smooth*	Very smooth*	Very smooth	Very smooth
Gelling Strength	Not too firm but usable	Slightly firm but usable*	Not too firm but usable	Not too firm but usable	Slightly firm but usable*	Slightly firm but usable*	Firm and usable	Firm and usable
Gelling time (min)	5-35*	2-30*	10-45*	5-40*	10-35*	5-30*	15-20	10-15
Friability	Less durable	less durable	less durable	less durable	Durable*	Durable*	Durable	Durable
Surface streaking	Rough	Rough	Rough	Rough	Fine*	Fine*	Fine	Fine
Color	Amber*	Amber*	Grey/ black	Grey/ black	Amber/light yellow*	Amber/light yellow*	Amber	Light amber
P^H	Acidic* 6.4	Acidic* 5.0	Acidic* 5.9	Acidic* 4.8	Acidic 6.3	Acidic 5.3	Acidic 6.6	Acidic (5.6)

Key: *(morphological parameters within range and comparable with control).

On addition of nutritive components of Nutrient and Sabouraud dextrose broths to the pulverized seeds of the 4 plants, the gelling activity was more significant compared to that of distilled water alone. Also, morphological properties of the gelled plants showed distinct variations (Table 5). D. microcarpum possessed relatively similar properties (smoothness, hardness, gelling strength, friability, surface streaking potential) than B. eurycoma and M. sloaneii compared to that of controls. From the solution, all the plants did not have homogeneous mix, which could be attributed to the presence of unwanted, seed coats and undissolved particles arising from incomplete polishing or grinding of the seeds and suitable sieving for powdery form The least available sieve size of 250 mm did not completely sieve or make a fine powder of the plants.

This also affected the clarity of the gelled products. Gelling time was within time limit and even less (5-50 minutes at 4.2 g and 8.4 g in 150 mL) compared to the controls (NA and SDA) (15-30 min at 4.2 g and 8.4 g in 150 mL) respectively. B. eurycoma, D. microcarpum and M. sloanei gelled based showed acidic pH. B. eurycoma, and D. microcapum at concentration of 0.028g/mL showed same pH level of 6.4±0.2 with that of NA except for M. Sloane. Whereas, at concentration of 0.065g/mL, all the plants possessed a stronger acidic pH of 5.0-5.9 equivalent to that of SDA (5.6). Those features with asterisk (*) showed significant compared to controls. Therefore, this showed that these plants could favour the cultivation of both bacteria and fungi at their respective pH levels and adjustments. The acidic pH of these plants is in line with previous publications.

Texture analysis is infinitely more descriptive of gel texture than simple gel strength measurements (Sanderson et al., 1989). This analysis reveals rupture strength, brittleness, toughness and adhesiveness of the media. Rupture strength is the maximum force required to rupture the gel and the total work required for the penetration of the probe for pre-adjusted distance and a constant speed is the measure of toughness of the gel (Chapman and Chapman, 1980). Whereas the distance that the probe penetrates in the gel before this rupture (break) occurs is indicative of the gel's elasticity (brittleness).

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Thus morphological properties could be sequenced as follow; Consistency: Similar to controls (NA and SDA); Transparency: control > B. eurycoma > D. microcapum > M. sloanei; Smoothness: Similar to that of control; Gelling Capacity: B. eurycoma >D. microcarpum > M. sloanei Gelling Time: Similar to that of Control; Friability: control > D. microcarpum > B. eurycoma > M. sloanei; Surface Streaking: control > D. microcarpum > B. eurycoma > M. sloanei; Colour: D. microcapum > B. eurycoma > M. sloanei; pH: D. microcarpum = B. eurycoma = M. sloanei (acidic medium).

The different plants-seeds with gelling property will be subjected to further research work to determine their exact composition compare to agar-agar which composed of agarose and agaropectin. Comparing agar, as an inert substance, which requires a nutritive component for growth of microorganisms, the seeds of Brachystegia eurycoma, Mucuna sloanei, and Detarium microcarpum, possessed both the gelling and its nutritive components as an added advantage (Onyeso, et al., 2016; Ndukwu, et al., 2017). But the aspect of bleaching with sodium hypochlorite seeks to render the seeds devoid of its constituencies.

CONCLUSION

The seeds of B. eurycoma, M. sloanei and D. microcarpum except C. mannii, possess relative gelling property at varying concentrations with some morphological parameters similar and others at variant when compared to agar as gelling agent for microbial culture media. This study also revealed that B. eurycoma, followed by D. microcarpum and M. sloanei exhibited significant gelling properties that are worth investigating further as possible alternative gelling agent to agar for formulation of microbial culture media.

RECOMMENDATIONS

The gelling and morphological properties of these plants are recommended for further research work to specifically determine the exact components in the plants seeds responsible for gelling and utilization in applicable fields. Further research work is recommended using different extraction techniques and or solvent systems, different species of these plants, sophisticated particle size reduction equipment for smooth polishing of pulverized plants-seeds, pH adjustment and in the usage of these plants seeds as alternative to agar in the formulation of microbial culture media.

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