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Functional and Swelling Properties of Galactomannan Hydrogel Extracted From Neem (*Azadiracta Indicia)* Stem Bark Obtained From Biu Local Government Area, Borno State, Nigeria

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Abstract: Neem stem bark is a galactomannan material having hydrogel properties because of its three-dimensional network leading to swelling propensity, viscosity, and binding properties which can be used for dual purpose of binder and enhancing medicinal properties of same cure. Hence, the objective of this work was to investigate the Functional and Swelling Properties of Galactomannan Hydrogel extracted from Neem (*Azadiracta indicia)* Stem Bark obtained from Biu Local Government Area, Borno State, Nigeria. The physicochemical, functional properties of the neem stem bark were analyzed to ascertain the active ingredient, the swelling properties were observed at distilled water, pH2.2 and 7.4 corresponding to the pH for large and gastrointestinal respectively. The functional and proximate analysis differs with geographical location taking account on the mineral composition of the soil. Hydrogel of 500 mm has the height swelling at (0 – 9 hours) of all the conditions showing absorption in the order of 6.5g, 3.9g, 5.2g and 2.6g relating the swelling percentage as NSB4<NSB3<NSB2<NSB1 at significant difference p<0.05 values all at the hour of 1-9 hours. This proves that the neem is also suitable as a binder

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Neem stem bark is a galactomannan material which possesses hydrogel properties Hydrogels can respond to external environmental changes because of their ability to swell in an aqueous media which does not dissolve in the aqueous media due to their three-dimensional networks (Cauich–Rodriguez *et* al., 2001; Baljit *et al.,* 2007). The factors responsible for the hydrogel changes to the external environment include PH, temperature, and ionic strength which enable them for wide applications such as PH-specific membrane separation, solution separation, solvent recovery, and pharmaceutical, etc. (Cauich-Rodriguez *et al*., 2001). The volume in which material responds to volume phase transition plays an

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important role in advanced technologies (Baljit *et al*., 2007a). Material with binding, water propensity behavior especially the volume phase transition has become a good potential for technological advancement (Baljit *et al.*, 2007a). Materials that are non-toxic, non-carcinogenic with thermal stability properties like Tamarind have been accepted by regulatory bodies for drug deliveries (Sumthi and Ray, 2002). Polymeric materials are also used in drug delivery because of their monomer arrangements that allow permeability and elasticity (Sumanthi and Ray, 2002: Tommansina *et al*., 2007). Konkoli seed gum (KSG) is a galactomannan material that possesses hydrogel properties, scientifically known as *maesopsis eminii*, in Tanzania is commonly called “Musizi” (Hall, 1995; Rugalema *et al.*, 1994: Joker, 2000). In Northeastern Nigeria is known as “Konkoli” it is used at home for beaking and soup thickener because of its three-dimensional network leading to swelling propensity, viscosity, and binding properties (Barminas and Eromosele, 2002; Osemeahon *et al.*,2008)

The swelling properties of the hydrogel are mostly affected by some factors such as the concentration of polymeric networks of the polymer, the swelling medium either distilled water or pH medium (Nkafamiya II *et al.*, 2011). There has been much research on polysaccharides due to their crosslinking properties for the development of hydrogel in drug delivery, these include carboxymethyl cellulose (Bajpai and Mishra, 2004; Baljit *et al.*, 2007a), starch (Bajpai and Saxena, 2004), Chitin (Mahdavinia *et al.*, 2004), dextran (Chiu *et al.*, 1999; Kim and Oh, 2005), guar gum (Gilko *et al.*, 1998; Prasad *et al.*, 1998; Xiuyu *et al.*, 2006) and psyllium (Baljit *et al.*, 2007a). Neem is botanically called *Azadirachta Indica*, many places have different ways of calling the plant commonly as neem, nimtree, or Indian lilac (Barstow and Deepu, 2018), in Nigeria especially northern Nigeria is called dogoyaro or dogonyaro (USDA, 2017).

The neem tree is botanically known as *Azadiracta indicia* categorized as the Mahogany family miliaceae, its roots penetrate deep into the soil, produce sucker when injured, and consists of green leaves, small flowers, seeds enclosed in a shell and kernel mostly yellowish or greenish, neem is usually identified by specific smell which is chemically identified for insecticide properties known as *Azadiractine* (Jarell, 1981). This research endeavor was initiated with the purpose of discovering a novel hydrogel that can augment the current hydrogels for drug delivery with dual purpose of being a binder and as well as serving as drug itself or enhancing the potential of the drug because of the medicinal/ biological properties of the neem stem bark. Drug delivery systems have the potential to attain more efficient therapies while simultaneously avoiding the risks of under and overdosing. Additionally, they facilitate the maintenance of drug levels within the desired range and reduce the necessity for frequent drug administration. Hence, the objective of this work was to investigate the Functional and Swelling Properties of Galactomannan Hydrogel extracted from Neem (*Azadiracta indicia)* Stem Bark obtained from Biu Local Government Area, Borno State, Nigeria

MATERIALS AND METHODS

Materials: Neem stem bark was obtained from Biu, a Local Government Area of Borno state. The stem bark was cleaned to remove dirt, shed dried for four weeks (30 days), and ground using mortar and pestles. The reagents used were of analytical grades which include Borax, HCL, KH_2PO_4, and NaOH.

Preparation of neem stem bark extracts: Extracts of ethanol, acetone and methanol were obtained using the works of [Halima et al 2008; Kokate et al., 2009]

Physicochemical analyses of neem stem bark: The Physicochemical analysis of the neem stem bark was carried out by the work of Biswa *et* al., (2002)

Mesh and particle size distributions (PSD): The mesh and particle size distributions of the ground neem stem bark in millimeter (mm) was adopted based ontheworkofIbrahim*etal.*, (2019)

$$\text{Absorption (\%)} = \frac{(W_2 - W_1)}{W_0} \times 100 \quad (1)$$

Where: W_1 = weight of the dry bag plus sample, W_2 = weight of the wet bag plus sample, W_0 = weight of the original sample

Preparation of buffer solution: Two different buffer solutions of pH7.4 and pH2.2 were prepared respectively. pH2.2 was prepared by adding 7.8 mL of 0.2 N HCl, 50 mL of 0.2 M KCl with distilled water to mark 200 mL of volumetric flasks, initially the 0.2 M KCl solution was prepared by dissolving 14.9 g of KCl in separate volumetric making 1000 mL with distilled water while the buffer solution of pH7.4 was prepared also adding of 39.1 mL of 0.2 N NaOH, 50 ML of 0.2 M KH_2PO_4 with distilled water to mark 200 mL of volumetric flasks while 0.2M KH_2PO_4 was prepared by dissolving 27.2 g of KH_2PO_4 with distilled water by marking 1000 mL of the volumetric flask. The pH 7.4 and 2.2 becomes applicable for the study of swelling behaviors of these hydrogels because they are the pH for large and gastrointestinal respectively (Orienti *et al*., 2001; Baljit *et al*., 2007b)

Fourier transforms infrared spectroscopy (FTIR): The modification nature of the Neem Seed after preparation into the hydrogel was studied using Fourier transform infrared spectroscopy showing both the Neem seed before and after the modification (FTIR 8400S Shimazu)

Statistical analysis: The statistical analysis was carried out using one-way analysis of variance (ANOVA) by Statistical Package for Social Science (SPSS) at significant level of P>0.05.

RESULTS AND DISCUSSION

Table 1 showed the proximate composition of three samples collected from different locations. NBS3 sample has a high moisture content compared to NSB1 and NSB2, while NSB1 has a high ash content compared to NSB2 and NSB3. NSB1 has a relative low content in fat while NSB3 is high in fat. The crude protein is high in NSB3 and low in NSB2, also having a high a high content of crude fiber in NSB1 and relatively low in NSB3 and the carbohydrate content found high in NSB1 and low in NSB2. Therefore, above analysis clearly indicated that samples from different geographical areas may not contain the same level of proximate composition due to the mineral compositions of the soil in the particular location.

According to Nwosu (2013), he reported that 10.87% moisture content was found in 100% stem bark of neem while 7.38% and 7.02% in neem leaves and 7.04% and 6.53% in Dioscorea dumentorum (bitter yam) leave was reported by Ogbuagu (2008). In the study on the proximate and functional properties of open air, solar and oven dried cocoyam leaves, by Amandikwa (2012) she reported moisture content values in the range of 6.5- 13.2 %. This shows that the moisture content of every food sample is equal to the quantity of solid matter in the sample, the higher the moisture present, the higher the rate of spoilage, as the rate of spoilage is closely related to the amount of moisture present (Sanful *et al*., 2013).

Table 1: Proximate composition of the neem stem bark collected from three different locations

Samples	Moisture	Ash	Fat	Crude protein	Crude Fiber	Carbohydrate
NSB1	5.12 ± 0.00	2.62 ± 0.01	2.91± 1.23	3.92 ± 1.33	1.12 ± 0.01	4.23 ± 0.16
NSB2	6.07 ± 0.01	2.04 ± 0.01	5.13±0.00	3.45 ± 0.00	1.02 ± 0.01	4.21 ±0.01
NSB3	6.78 ± 0.01	2.34 ± 0.00	5.23±0.00	4.34 ± 0.00	1.00 ± 0.00	4.22 ± 0.14

Table 2: Functional properties of neem stem bark obtained from three different locations

Sample	Bulk density (g/cm^3)	Water absorption capacity (g/g)	Swelling index (g/g)	Gelation (^0C)	Wettability (Secs)	Viscosity (CP)
NSB1	0.51 ± 0.00	6.51 ± 0.03	1.07 ± 0.00	40.03±0.00	1.07±0.00	1.200E±1.00
NSB2	0.52 ± 0.00	4.11 ± 0.02	1.25 ± 0.00	40.04±0.00	1.03±0.00	1.200E±1.00
NSB3	0.51 ± 0.00	2.21 ± 0.00	1.08 ± 0.00	40.01±0.00	1.29±0.00	1.200E±1.00

Table 3: Qualitative phytochemical analyses of acetone, ethanol and methanol extract of *azadirachta indica* stem bark

Solvent used for the extraction	Alkaloid	Reducing Sugar	Flavonoid	Saponins	Tanin	Volatile oil	Glycoside	Terpenoid
Acetone (A)	+	+	+	+	+	+	+	-
Methanol (M)	-	+	-	-	-	-	+	+
Ethanol (E)	-	+	+	+	+	-	-	-

KEY: +++ Appreciable amount; ++ Moderate amount; + Trace amount; - Not Detected (absent)

Table 3 shows the presence of secondary metabolites in neem stem bark using three different solvents for the extraction (acetone, methanol and ethanol). The prominent metabolites in the neem stem bark are reducing sugar in appreciable amount, flavonoid, saponins, tannin and glycoside in moderate amount while alkaloid, volatile oil and terpenoid in trace amount. Alkaloid, Saponins aids the reduction of blood cholesterol level, reduce the risk of cancer and stimulate our immune system. It also aids the treatment of certain diseases such as malaria, eczema etc. and can also be used as a detergent, thus, it is economical when compared to other chemical detergents. Saponins are widely used also for their effects on ammonia emissions in animals (Hostetman and Marston, 1995)

Table 4 shows the percentage of the secondary metabolite present in the neem stem bark extracted using methanol, ethanol and acetone, the results shows that Alkanoid has the highest percentage followed by volatile oil when extracted with acetone, highest percentage of methanol was investigated in Terpenoid, the lowest percentage of the acetone, methanol and ethanol was indicated in reducing sugar phytochemical analysis. Proximate analysis of some leaves such as moisture, protein, lipid, carbohydrate and ash content decreased in value when dried (Ibrahim *et al*., 2019). Temperature has significant effect on the nutrient content of plants (Ibrahim *et al*., 2019). Table 5 shows the mesh and particle size distribution of the grinded neem stem bark in the ranges of 500 - 700 mm mesh sizes to 0.90 – 2.15 mm particle size distribution for the purpose of swelling in distilled water, pH values corresponding to the large and small intestines. Varying the adsorbent particle sizes allowed the difference in adsorption to be correlated (Prashanth *et al*., 2019). Figure 1a, b and c the Swelling properties of the various mesh sizes of the neem seed galactomannan hydrogel which was analyzed using 2.6g/2.6g that is 1:1 ratio of Neem seed powder and Borax (1:1 glactomannan hydrogel) under the condition of distilled water and both pH7.4 and pH2.2 respectively. The results shows that the glactomannan hydrogel of 500 mm has the highest swelling properties (percentage) at all the durations (0 – 9 hours) indicating both in distilled water, pH7.4 and pH2.2 respectively this has to do with the surface area of the material. Properties of material (absorption, adsorption and catalytic) increases with increasing the surface area of the material (Arafat and Akbar, 2014). It has been reported that the surface area of materials mostly decreases with increase in the molar ratio of the active component on the support material (Kostedt *et al.*, 2010).

Table 4: Quantitative estimate (mean) of the secondary metabolites present in the neem stem bark

Phytochemical	Mean (%)
Alkanoid	0.4912
Reducing Sugar	0.0700
Flavonoid	0.7295
Saponins	0.0055
Tanin	0.3455
Volatile oil	0.3954
Glycoside	0.2954
Terpenoid	0.2340

Table5: Mesh and particle size distribution of grinded neem stem bark (nbs3)

S/N	Mesh size (mm)	PSD (mm)
1	700	2.15
2	650	2.10
3	600	2.05
4	550	1.80
5	500	0.90

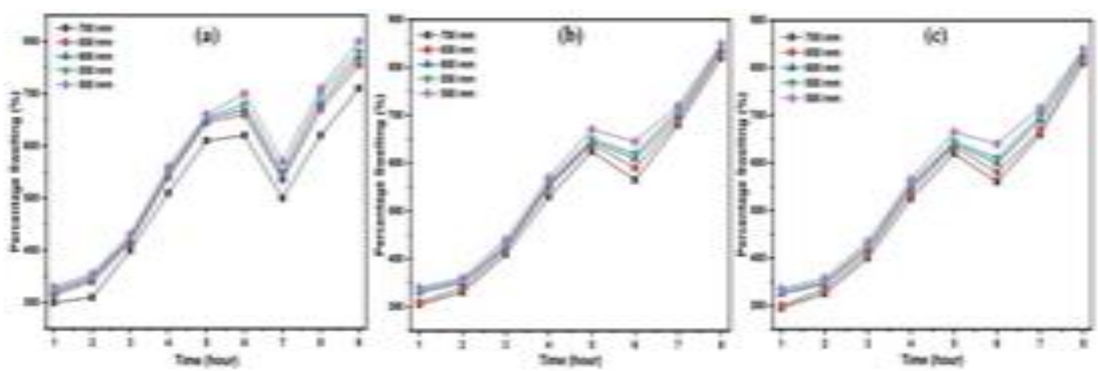

Fig. 1a, b, c: Effects of distilled water (%), pH7.4 and pH2.2 on various mesh sizes of the neem seed blend

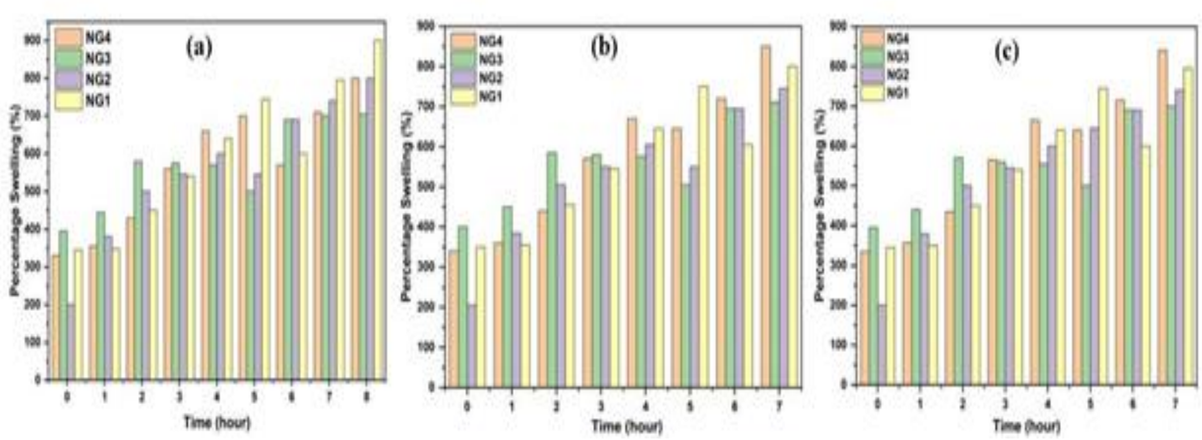

Fig. 2a, b, c: Effect of increasing NG on swelling (distilled water %, pH4.7 % and pH2.2 %)

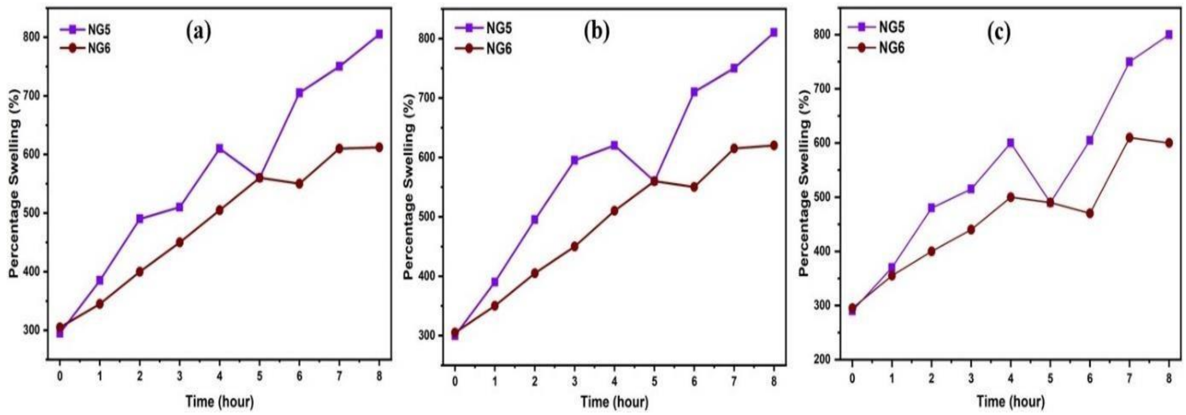

Fig. 3a, b, c: Effect of increasing borax on swelling (distilled water %, pH7.4 % and pH2.2%)

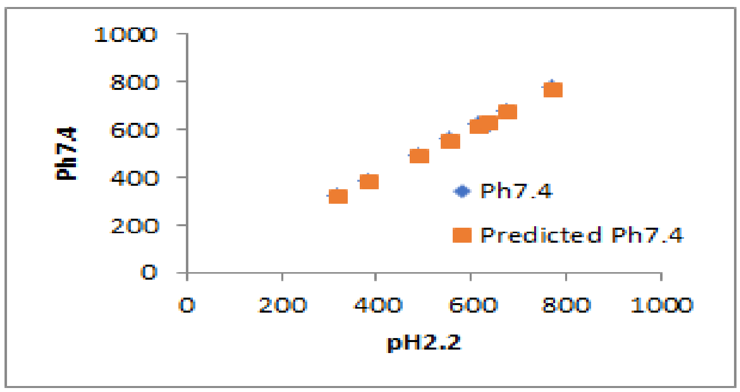

Fig. 4: Relationship between the pH 7.4 and pH 2.2

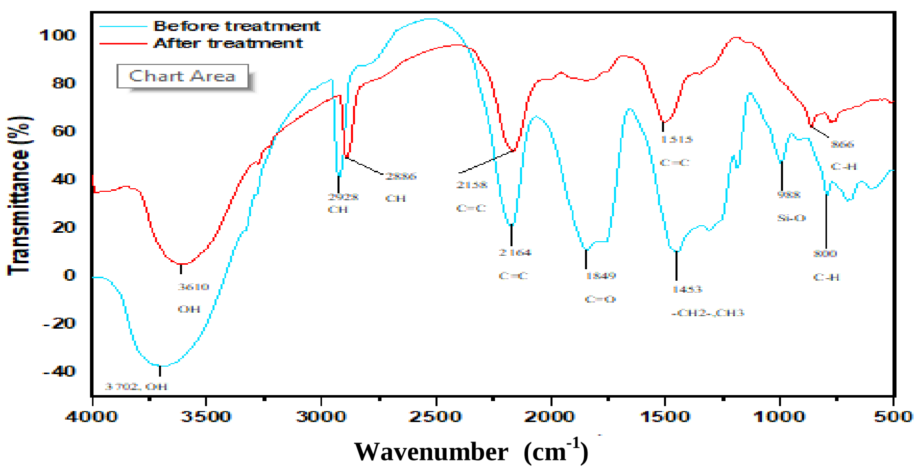

Fig. 5: Fourier Transform Infrared Spectroscopy (FTIR) of Neem Before and After Treatment

Figure 2a, b and c indicate the swelling behavior of the neem seed hydrogels on constant crosslinking agent (borax) at 2.6g with increasing the concentration of the neem seed galactomannan (NG) ranging from 2.6g, 3.9g, 5.2g to 6.5g in distilled water, pH7.4 and 2.2 respectively. The result shows that the absorption of water, pH solution (7.4 and 2.2) increases with the increase in the concentration of the galactomannan, the absorption is in the order of 6.5g, 3.9g, 5.2g and 2.6g galactomannan this is simply because the structure of the swelling material becomes loss (less tight) on the increase of the galactomannan over the crosslinking agent (borax) thereby giving more room for the absorption. The swelling behavior of hydrogel is directly related to the loading and release of drug from the polymeric metrics, the mechanism or kinetic of swelling properties of a polymer is based on the internal makeup of the polymer that is the crosslinking properties of the polymer matrix which has the ability of making the polymer to be stiff or elastic depending on the crosslink ratio for an instance the more the crosslinked the polymer the less will it respond to environmental changes (swelling and releasing properties) compared to same polymer with less crosslinking agents (Baljit *et al*., 2007a). All the prepared hydrogels demonstrated good absorption properties of distilled water and the pH values, but 6.5/2.6(w/w) galactomannan/borax ratio swells more by having a higher percentage of swelling at the duration of 5 -9 hours. The swelling percentages of the hydrogels were in the order of NSB4<NSB3<NSB2<NSB1 which has significant swelling difference at $p<0.05$ values. The hydrogels also demonstrated an increasing trend in water absorption over the course of 1-5 hours, followed by a decrease at the 6-hour mark. Subsequently, there was a rise in water absorption from 7-9 hours, apart from NSB1, which displayed a decrease at the 7-hour mark and then an increase from 8-9 hours. The observed phenomenon can be attributed to the relatively lower concentration of neem stem bark in the structural components denoted as NSB2, NSB3, and NSB4, as compared to that in NSB1. Among the various hydrogels examined, it has been determined that the hydrogel formulated with a weight-to-weight ratio of 6.5/2.6 exhibits superior performance. This is evidenced by its ability to absorb a greater amount of water and its network structure beginning to degrade after 7 hours, as opposed to 6 hours for the other hydrogels. These findings suggest that NSB1 would be the optimal choice for achieving superior water uptake, followed by NSB2.

Figure 3a, b and c show the outcomes of the experiment involved the elevation of concentration of BX while maintaining Neem stem bark concentration constant. Figure NG5 demonstrated a higher level of water absorption than NG6 ($P < 0.05$). This can be attributed to the fact that NG6 has a higher concentration of the crosslinking agent, thereby resulting in a compact structure and less swelling. At the 7th hour, NG5 began to lose its network structure, while KG6 started to lose its structure at the 6th hour, indicating that NG5 is better in terms of swelling and potential drug delivery. Figure 4: Fourier Transform Infrared Spectroscopy (FTIR) is often used to identify unknown compound or confirm the identity of known compounds. It can also be used to determine the purity of a compound, as well as its physical and chemical properties. The figure represents the functional groups of pure (raw) neem sample and the isolated neem extracts using Fourier Transform Infrared Spectroscopy. The respective wave number (inverse of wavelength cm^{-1}) of each peak were assigned to the fingerprint region (1500-500 cm^{-1}), the functional group region (1500-4000 cm^{-1}), and the OH/NH stretching region (4000-5000 cm^{-1}). The specific peak of energy at a given wavenumber can shift due to other chemical and matrix factors (as well as by the way the incident energy is introduced). Also, the two figures give the difference (additional, removal) of the functional groups between the raw and treated samples. Frequency range of 4000 – 3000 cm^{-1} assigned to various absorption of 3550-3200 $cm^{-1}$for both the raw and treated sample, appearance as strong, broad, group OH stretching, class of compound alcohol which is intermolecular bonded, frequency range of 3000-2500 cm^{-1} assigned to absorption band of 2400-2000 cm^{-1} strong appearance, grouped as O=C=O stretching, compound classified as carbon dioxide, frequency range of 2000-1650 cm-1 assigned to absorption band of 1847.28 cm-1 appeared strong C=O stretching group and classified as anhydride compound. The absorption band 1411.94 and 1134.18 cm^{-1} appeared to be sharp and broad in the isolated neem extracts unlike the raw neem sample which can be categorized as C-H bending alkane group specifically methyl and methylene groups. The major difference between the raw and the treated sample is the finger print regions with the frequency range of 900 – 700 cm^{-1} absorption band of 759.98 to 455.22 cm^{-1} of absorption band appeared strong, grouped as C- Br stretching and C-I stretching classified as halo compound, this implies that finger print region was introduced in to the sample after the treatment. Figure 5 is the statistical graph. The R^2-value of Microsoft excels and statistical package for social science (SPSS) were 0.964 and 0.995 of the various neem glactomannan hydrogel at constant crosslinking agent this implies that there is significant difference between the swelling behaviors of the various prepared hydrogels in distilled water. Likewise, the swelling behavior proves significant difference of the various hydrogels in pH7.4 and 2.2 with p-values of 0.951, 0.958 and 0.983, 0.999 of Microsoft excel and SPSS respectively, therefore the population is normally distributed. R value, 0.997 of regression and correlation using Microsoft Excel signifies that, there is a strong relationship between the swelling properties of various hydrogel in Ph7.4 and2.2 respectively.

Conclusion: The results of the analysis proves that neem has properties which swell at the adequate amount of distilled water and pH at the required ranges of time therefore it can act as a binder and also enhance the medicinal properties of the material its binding because itself is a medicinal plant but the functional and proximate properties of the neem differ with geographical locations.

Declaration of Conflict of Interest: The authors declare no conflict of interest

Data Availability: Data are available upon request from the first author or corresponding author or any of the others

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